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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A01N 43/04, 63/00, A61K 31/70, 48/00, C12N 15/00

A1

(11) International Publication Number: WO 95/34202

(43) International Publication Date: 21 December 1995 (21.12.95)

(21) International Application Number: PCT/US95/07554

(22) International Filing Date: 14 June 1995 (14.06.95)

(30) Priority Data:

08/260,201	15 June 1994 (15.06.94)	US
08/278,282	21 July 1994 (21.07.94)	US

(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

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(54) Title: METHODS FOR REDUCING HYPERACUTE REJECTION OF XENOGRAFTS

(57) Abstract

A method for reducing xenograft rejection is provided wherein a vector directing the expression of a protein having glycosyltransferase activity is introduced into xenogeneic cells. Cells so prepared exhibit a substantial reduction in their binding to naturally occuring preformed human antibodies and are thus protected from hyperacute rejection.

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METHODS FOR REDUCING HYPERACUTE REJECTION OF XENOGRAFTS

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of copending U.S. Patent Application Serial No. 08/260,201 filed June 15, 1994.

FIELD OF THE INVENTION

This invention relates to xenotransplantation. More specifically, the invention relates to methods that will prevent or reduce hyperacute rejection of xenogeneic cells, tissues and organs following transplantation into human recipients. The invention provides methods for stably reducing the expression on the surface of a xenogeneic cell of the non-human antigen known as galactose $\alpha(1,3)$ galactose. This prevents the phenomenon of antibody-dependent rejection of xenogeneic cells that typically follows exposure to human blood, plasma, or serum (e.g., following xenotransplantation into a human patient) as a result of the binding of preformed natural human antibodies to the surfaces of such cells.

BACKGROUND OF THE INVENTION

Xenotransplantation: Surgical problems related to the transplantation of allogeneic organs (i.e., organs from donors of the same species as the transplant recipient), such as kidney, liver, heart, lung and pancreas, have been largely solved, and immunosuppression has been improved such that these procedures are now routinely performed with a high degree of success (Brent, 1991). However, a major problem in transplantation medicine today is the provision of sufficient allogeneic donor organs to satisfy the large numbers of patients awaiting a transplant. Given the increasing emphasis on the costs of dialysis and hospitalization incurred by

patients awaiting transplantation, there is even greater emphasis on the transplantation of donor organs early in the course of disease. Additionally, it is clear that the supply of human donor allografts cannot satisfy this demand. Alternative sources for replacing diseased organs, tissues, or cells, are mechanical devices or animal organs. All clinical transplantations of animal organs have met with failure other than when closely related Old World primate species, such as, baboon, chimp or gorilla, were used as donors. Unfortunately, the supply of potential Old World primate donors is also limited, and ethical considerations further limit the use of organs from such species. Non-primate species, on the other hand, offer a vast potential source of donors.

The most likely donor species for xenotransplantation appears to be the pig (Cooper et al., 1991 and Niekrasz, et al., 1992). This animal is commonly used commercially, and therefore its use will engender fewer ethical problems than the use of primate donors. Furthermore, the pig is considered a highly suitable donor for anatomical and physiological reasons (Cooper et al., 1991 and Niekrasz, et al., 1992).

Immunological Rejection of Xenografts: The rejection of transplanted cells, tissues, or organs may involve both an extremely rapid hyperacute rejection (HAR) phase and a slower cellular rejection phase. HAR of non-human, non-Old World primate organs, tissues, or cells (referred to herein as "xenogeneic" organs, tissues, or cells, or "xenotransplants", or "xenografts") is initiated by preformed natural antibodies found in human blood, plasma, serum, lymph, and the like, that bind to donor cells, e.g., endothelial cells, and activate attack by the complement arm of the human immune system (Dalmasso, et al., 1992; and Tusso, et al., 1993).

While some xenograft tissues (e.g., porcine pancreatic islets) do not appear to be rejected by this mechanism, HAR is the most significant impediment to the

successful xenotransplantation of most cells and tissues, and of all vascularized organs. Methods for the control of the HAR are available. These include interference with the antibody antigen reactions responsible for initiating the HAR response, either by removing the preformed natural antibodies from the circulation or by interference with the binding of the natural antibodies to their specific epitopes (see copending U.S. application Serial No. 08/214,580, entitled "Xenotransplantation Therapies", filed by Mauro S. Sandrin and Ian F.C. McKenzie on March 15, 1994, and PCT publication No. 93/03735, entitled "Methods and Compositions for Attenuating Antibody-Mediated Xenograft Rejection").

A particularly desirable approach to the prevention of hyperacute rejection is to delete or inhibit the $\alpha(1,3)$ galactosyltransferase gene in xenogeneic cells, and to thus eliminate or significantly reduce expression of Gal $\alpha(1,3)$ Gal epitopes on the surface of such cells (see copending U.S. patent application Serial No. 08/214,580, supra). This approach eliminates or reduces the binding of preformed natural human antibodies to the xenogeneic cells and, therefore, prevents or reduces the activation of complement and subsequent hyperacute rejection of xenogeneic cells, tissues and organs.

Inhibition of complement attack on the xenotransplant may be accomplished by several means, including the use of complement inhibitors such as the 18kDa C5b-9 inhibitory protein and monoclonal antibodies against human C5b-9 proteins as disclosed in U.S. Patent No. 5,135,916, issued August 4, 1992.

The foregoing methods are effective, but have certain drawbacks in practice, potentially requiring the continuous administration of pharmacologic agents, or, in some cases, requiring the technically difficult production of animals carrying a targeted disruption of a specific gene.

HAR and Complement: Activation of complement leads to the generation of fluid phase (C3a, C5a) and membrane bound (C3b and C5b-9) proteins with chemotactic, procoagulant, proinflammatory, adhesive, and cytolytic properties (Muler-Eberhard, 1988). Immunohistological analysis of hyperacutely rejected xenotransplants reveals antibody deposition, complement fixation, and vascular thrombosis as well as neutrophil infiltration (Zehr, et al., 1994; Auchincloss, 1988; Najarian, 1992; Somerville and d'Apice, 1993; and Mejia-Laguna, et al., 1972).

HAR and Xenoantigens: The targets of natural human antibodies have been the subject of investigations for a number of years, as the identification of these xenoantigens would enable the development of strategies to circumvent hyperacute rejection of xenografts. Several recent studies have convincingly demonstrated that the carbohydrate galactose $\alpha(1,3)$ galactose (Gal $\alpha(1,3)$ Gal) is the major xenoepitope recognized by natural human antibodies (see Sandrin, et al., 1993A; Sandrin, et al., 1993B; copending U.S. patent application Serial No. 08/214,580, supra; and PCT publication No. 93/03735, supra).

Galili and colleagues have shown that a large proportion of IgG (1%) in human serum is directed against the Gal $\alpha(1,3)$ Gal epitope expressed as part of a variety of glycosylated molecules found on both cell surfaces and on secreted glycoproteins (Galili et al., 1984; and Thall and Galili, 1990). This disaccharide epitope is found in all mammals except humans and Old World primates, and naturally occurring preformed anti-Gal $\alpha(1,3)$ Gal antibodies are found only in humans and Old World primates, i.e., those species which do not themselves express the epitope (Galili et al., 1987 and Galili et al., 1988).

HAR and Preformed Natural Antibodies: The immunoglobulin class of an anti-Gal $\alpha(1,3)$ Gal antibody determines the biological role of that antibody in

hyperacute rejection. On the basis of histological studies, Bach and Platt (Platt et al., 1990; Platt and Bach 1991; Platt et al., 1991; and Geller et al., 1993) consider that IgM is the most important class of immunoglobulin involved in hyperacute xenograft rejection.

However, natural human antibodies to Gal α(1,3) Gal are not exclusively of the IgM class, and several studies demonstrate the presence of IgG antibodies reactive with pig cells in human blood (Tusso et al., 1992; Fabian et al., 1992; Hammer et al., 1992; Cairns et al., 1993A; Cairns et al., 1993B; Fournier et al., 1993; Koren et al., 1993; and Zhao et al., 1993), in agreement with the original findings of Galili, et al., 1984 (see also Galili, 1993). For example, by eluting antibodies from different xenogeneic organs after perfusion with normal human serum, Koren et al., 1992, have demonstrated the presence of IgM, IgG and IgA antibodies. Based on these various studies, there is a general consensus that both IgM and IgG antibodies react with Gal α(1,3) Gal antigens.

The ability of different monosaccharides and oligosaccharides to inhibit the interaction of naturally occurring preformed human antibodies with pig cells and to prevent the antibody-dependent and complement-mediated lysis of pig cells has been examined (Sandrin et al., 1993A; Sandrin et al., 1993B; PCT publication No. 93/03735, supra; and copending U.S. patent application Serial No. 08/214,580, supra).

Inhibition of the binding of such antibodies to xenogeneic cells was obtained with galactose, or with moieties containing terminal galactose in an α linkage but not a β linkage. Various carbohydrates have also been shown to contain the target epitopes for several types of naturally occurring preformed human antibodies with other specificities (e.g., ABO blood group antibodies). However, no monosaccharide tested, other

than those containing the Gal α(1,3) Gal epitope, had any inhibitory effect on the binding of naturally occurring preformed human antibodies to xenogeneic cells. Identical inhibition results were obtained when individual human serum samples from blood group A, B, AB or O individuals were used (Sandrin et al., 1993A and Sandrin et al., 1993B).

Similarly, Cooper and colleagues have demonstrated that, of a total of 132 carbohydrates screened for binding to preformed naturally occurring human IgG and IgM antibodies, each of the four carbohydrate molecules that they found could bind such antibodies contained a terminal α galactose (Good et al., 1992). The four carbohydrates were:

(1) Gal α(1,3) Gal β(1,4) GlcNAc,

(2) Gal α(1,3) Gal β(1,4) Glc,

(3) Gal α(1,3) Gal β, and

(4) Gal α(1,3) Gal.

Sugars such as melibiose (a disaccharide containing a terminal galactose in an α linkage) coupled to a carrier such as SEPHAROSE can be used to purify anti-Gal α(1,3) Gal antibodies (Galili et al, 1984 and Galili et al., 1985). In some antibody absorption experiments, human serum was passed over the carrier-sugar matrix in order to prepare serum from which the antibodies reactive with the sugar were removed. The results of testing the cytolytic activity of the sera prepared in these experiments indicate that the majority of the cytotoxic antibodies were removed from the serum by these means (Sandrin et al., 1993A; Sandrin et al., 1993B).

In sum, the results of the sugar inhibition studies, the studies of the binding of antibodies to terminal α galactose-containing molecules, and the studies of the absorption of antibodies by melibiose-SEPHAROSE, all lead to the conclusion that Gal α(1,3) Gal epitopes are the

most important epitopes detected by naturally occurring human IgG and IgM antibodies.

Glycosyltransferases

Mammalian cells display a complex variety of carbohydrate antigens on their surfaces. Carbohydrate epitopes are expressed on all mammalian cells by membrane glycoproteins and glycosphingolipids. Profound changes in the structures of these glycoconjugates frequently accompany important biological processes such as differentiation and development. The types and numbers of carbohydrate epitopes present on cells vary in different species and in different tissues within a given species (Yamakawa and Nagai, 1978).

The structures of these carbohydrate moieties are determined largely by the activities of the glycosyltransferases responsible for oligosaccharide synthesis. Therefore, the population of oligosaccharide molecules displayed on the surface of a given mammalian cell is largely determined by the repertoire of glycosyltransferases active in the cell (Kornfeld and Kornfeld, 1985).

The glycosyltransferases comprise a family of enzymes that transfer sugars from nucleoside diphosphate-sugar conjugates (donor molecules) to acceptor substrate molecules, forming covalent linkages. Acceptor substrates are often oligosaccharides or oligosaccharide moieties of larger molecules, but may also be specific proteins or lipids. Glycosyltransferases function in a sequential manner, such that the oligosaccharide product of a transferase activity often becomes the acceptor substrate for subsequent transferase activity. The final result generally contains a linear and/or branched polymer of component monosaccharides linked to one another.

Glycosyltransferases differ from each other with respect to the nature of the nucleoside diphosphate-carbohydrate donor, the nature of the acceptor substrate,

and the glycosidic linkage joining the donor sugar to the acceptor substrate (reviewed by Beyer and Hill, 1982). Examples of glycosyltransferases include the following: galactosyltransferases, fucosyltransferases, sialyltransferases, N-acetylglucosaminyltransferases, N-acetylgalactosaminyltransferases, glucosyltransferases, sulfotransferases, acetylases, and mannosyltransferases.

Galactosyltransferases

The galactosyltransferases are examples of glycosyltransferases that transfer galactose from a UDP-galactose donor molecule to an acceptor substrate. One such galactosyltransferase, UDP-Gal:Gal β(1,4) Gal NAcGlc α(1,3) galactosyltransferase (also referred to as α(1,3) Gal transferase), is a Golgi membrane-bound enzyme that catalyzes the following reaction:

UDP-Gal + Gal {β(1,4) or β(1,3)} Glc NAc-R → Gal α(1,3) Gal {β(1,4) or β(1,3)} Glc NAc-R + UDP

in which R may be a glycoprotein or a glycolipid (Blanken and Van den Eijnden, 1985). The resulting α(1,3) linked galactose occupies the terminal non-reducing position in N-acetyllactosamine-type carbohydrate chains and, as such, is a non-charged alternative to chain termination by sialic acid. As discussed above, such α(1,3) Gal structures are the most important epitopes of xenogeneic cells recognized by naturally occurring preformed human antibodies.

α(1,3) Gal transferase and the Gal α(1,3) Gal β1-R (herein referred to as Gal α(1,3) Gal) product of the activity of this enzyme show both species and tissue-specific expression (Galili et al., 1988). The α(1,3) Gal transferase is widely expressed in a variety of mammalian species, with the notable exception of Old World primates and humans. These mammals do not express the enzyme due to frameshift and nonsense mutations in their genomic sequences encoding this enzyme (Larsen et al., 1990a).

It is believed that humans and Old World primates have high levels of circulating natural preformed antibodies that bind specifically to the Gal α(1,3) Gal epitope as a consequence of these mutations and the resultant lack of the epitope in humans and Old World primates. The source of antigen exposure responsible for the natural preformed antibodies in these species has not been definitively established, but is believed to be certain bacteria bearing Gal α(1,3) Gal epitopes that are normally found in the intestines of humans and Old World primates.

The cDNA encoding the pig α(1,3) galactosyltransferase has been cloned using cross species hybridization (see copending U.S. patent application Serial No. 08/214,580, supra; and Dabkowski et al., 1993). Sequence comparison shows that at the amino acid level there is approximately 75% identity with the murine and approximately 82% identity with the bovine α(1,3) Gal transferase sequences, with the catalytic domains of the transferases having the highest identity.

Fucosyltransferases

The carbohydrate antigens and glycosyltransferases of the human H blood group, as well as the specific details of the biosynthesis and distribution of the H antigen, have been extensively reviewed (see, for example, Lowe, 1991). Several carbohydrates, including those associated with the H antigen, contain the terminal structure Fucose α(1,2) Galactose. The synthesis of the Fucose α(1,2) linkage is catalyzed by specific α(1,2) fucosyltransferase enzymes. The enzymatic activities of these transferases result in the covalent attachment of L-fucose by an α(1,2) linkage to a variety of acceptor molecules. The H transferase, for example, is a fucosyltransferase that catalyzes a transglycosylation reaction covalently linking a fucose to a specific oligosaccharide acceptor substrate. In this reaction the fucose is derived from the nucleotide sugar donor

molecule GDP-fucose and connected by an α(1,2) linkage to the Galactose residue of Gal β(1,3) GlcNAc-R or Gal β(1,4) GlcNAc-R acceptor substrates (i.e., galactose linked to N-acetylglucosamine in a β(1,3) or a β(1,4) linkage, where R represents a glycoprotein, protein, glycolipid, or lipid).

These acceptor substrates are also the acceptor substrates for the α(1,3) Gal transferase discussed above, although each transferase utilizes a different nucleotide sugar donor molecule (UDP galactose for α(1,3) Gal transferase vs. GDP fucose for H transferase). The α(1,3) Gal transferase and the H transferase have now been cloned (see copending U.S. patent application Serial No. 08/214,580, supra; Stanley, 1992; and Lowe, 1991). The recombinant expression of various glycosyltransferases, including the H transferase, in cells that would be expected to be expressing the α(1,3) Gal transferase has been reported (see, for example, Lowe, 1991). However, prior to the present invention, the effects of such recombinant expression on the expression of the Gal α(1,3) Gal epitope have been unknown.

SUMMARY OF THE INVENTION

In view of the foregoing, it is an object of this invention to provide genetically modified xenogeneic organs, tissues, and cells that are less prone to hyperacute rejection when exposed to human blood, plasma, serum, lymph, or the like (e.g., following xenotransplantation into human patients) than their unmodified precursors, and to provide methods for the preparation of such xenogeneic organs, tissues, and cells. In accordance with these methods, xenogeneic cells are genetically modified so that they express the glycosyltransferase activity of an exogenous glycosyltransferase (i.e., a glycosyltransferase encoded by a recombinant nucleic acid molecule introduced into

the xenogeneic cells or a parent cell of the xenogeneic cells). In particular, the genetically-modified xenogeneic cells of the invention exhibit reduced levels of the xenoantigen Gal α(1,3) Gal on their cell surfaces.

In another aspect of the invention, the genetically-modified xenogeneic cells of the invention are inhibited from binding to preformed naturally occurring human antibodies and are therefore significantly less sensitive to HAR as demonstrated by reduced sensitivity to activation and/or lysis by human complement. In this way, when transplanted into human patients, the rejection of such cells by complement-mediated hyperacute rejection mechanisms is reduced or prevented.

In certain preferred embodiments, the invention provides a method for reducing rejection of a xenogeneic cell following transplantation into a human or an Old World primate comprising:

(a) producing a genetically altered cell by introducing an expression vector comprising a nucleic acid sequence encoding a protein having fucosyltransferase activity into a recipient cell, the introduction of said expression vector causing a substantial reduction in the binding of naturally occurring preformed human antibodies or naturally occurring preformed Old World primate antibodies to the genetically altered cell when compared to the binding of said antibodies to the recipient cell; and

(b) transplanting said genetically altered cell or a cell derived from said cell into a human or an Old World primate.

In other preferred embodiments the invention provides an ungulate cell which has been genetically altered by the introduction of an expression vector comprising a nucleic acid sequence encoding a protein having fucosyltransferase activity into a recipient ungulate cell, the introduction of said expression vector causing a substantial reduction in the binding of

naturally occurring preformed human antibodies or naturally occurring preformed Old World primate antibodies to said genetically altered ungulate cell when compared to the binding of said antibodies to the recipient ungulate cell.

In further preferred embodiments, the invention provides a retroviral packaging or producer cell which has been genetically altered by the introduction of an expression vector comprising a nucleic acid sequence encoding a protein having fucosyltransferase activity into a recipient cell from which the genetically altered retroviral packaging or producer cell is derived, the introduction of said expression vector causing a substantial reduction in the binding of naturally occurring preformed human antibodies or naturally occurring preformed Old World primate antibodies to said genetically altered retroviral packaging or producer cell when compared to the binding of said antibodies to the recipient cell from which the genetically altered retroviral packaging or producer cell is derived.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1-4 are photomicrographs of African Green Monkey COS cells which have been fluorescently stained with anti-H antigen mAbs (FIGS. 2 and 3) or with lectins specific for the Gal α(1,3) Gal epitope (FIGS. 1 and 4). In each figure, the bottom panel shows all cells, as seen by phase contrast illumination, and the top panel shows only those cells specifically binding to the mAb or lectin as seen by ultraviolet illumination. The cells in FIG. 1 have been transfected with a vector expressing the Gal α(1,3) Gal transferase; the cells in FIG. 2 have been transfected with a vector expressing H transferase; and the cells in FIGS. 3 and 4 have been transfected with equal amounts of both vectors. African Green Monkeys are Old World primates and thus their cells, including COS cells, do not express the Gal α(1,3) Gal epitope. In addition, COS cells do not express the H epitope.

FIG. 5 illustrates the expression of the H epitope and reduced expression of the Gal α(1,3) Gal epitope in stably transfected porcine kidney cells as analyzed by lectin staining and fluorescence-based flow cytometric analysis of the cells.

FIG. 6 demonstrates the loss of human serum IgG and IgM binding to porcine kidney cells associated with the expression of the H epitope and reduced expression of the Gal α(1,3) Gal epitope as analyzed by fluorescence staining and flow cytometry.

FIG. 7 illustrates the enhanced resistance to human serum lysis associated with the expression of the H epitope and consequent reduced expression of the Gal α(1,3) Gal epitope in stably transfected porcine kidney cells.

The foregoing drawings, which are incorporated in and constitute part of the specification, illustrate the preferred embodiments of the invention, and together with the description, serve to explain the principles of the invention. It is to be understood, of course, that both the drawings and the description are explanatory only and are not restrictive of the invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Glycosyltransferases: A variety of nucleic acid molecules encoding glycosyltransferases can be used in the practice of the present invention provided that the glycosyltransferase encoded by the nucleic acid molecule is able to reduce the levels of Gal α(1,3) Gal epitopes on the surface of a xenogeneic cell in which the exogenous transferase is expressed. In accordance with the present invention, this property can be determined by introducing an appropriate expression vector directing the expression of the candidate glycosyltransferase into xenogeneic cells and then testing the cells for cell surface levels of the Gal α(1,3) Gal epitope using, for example, human serum, as described below in Example 4.

Transferases suitable for use in the methods and cells of the present invention will cause a substantial reduction in the binding of naturally occurring preformed human antibodies to the xenogeneic cells after introduction of the expression vector compared to binding before introduction of the vector. An at least 50% reduction in binding will, in general, comprise a "substantial reduction". Smaller reductions in binding are also considered "substantial" if they represent a statistically significant reduction, i.e., a reduction that, when analyzed by a standard statistical test, such as the student's T test, will give a probability value, p, less than or equal to 0.05 and, preferably, less than or equal to 0.015. Examples of the construction of such vectors, production of such cells, and the testing of such cells for reduction of preformed natural antibody binding are given below in Examples 1-5.

In particular, reduction in the binding of naturally occurring preformed antibodies can be determined by staining and counting stained cells as described below in Example 2, or by FACS analysis as described in Example 4 below in which case a quantitative readout can be obtained by measuring the areas under the various FACS curves and the shifts in the positions of those curves, or by measurement of changes in complement resistance as described in Example 5 below.

While not wishing to be bound by any particular theory of operation, it is believed that glycosyltransferases, which are able to reduce the levels of Gal α(1,3) Gal epitopes on the surface of a xenogeneic cell in which the transferase is expressed, effect this reduction by competition for a shared acceptor substrate. Specifically, it is believed that transferases suitable for use in the methods and cells of the invention transfer donor sugars to β(1,3) Glc NAc-R or β(1,4) Glc NAc-R acceptor substrates. Thus, preferred transferases include those that transfer donor sugars to β(1,3) Glc

NAc-R or β(1,4) Glc NAc-R acceptor substrates and create covalent linkages other than the Gal α(1,3) Gal linkage upon such transfer.

Preferred transferases to be used in the practice of the invention include fucosyltransferases. With regard to these transferases, it is believed that the addition of a terminal fucose residue to the β(1,3) Glc NAc-R or β(1,4) Glc NAc-R acceptor substrate of the α(1,3) galactosyltransferase prevents the addition of a Gal α(1,3) Gal epitope to the acceptor substrate. Specific examples of fucosyltransferases that can be tested for use in the process of the present invention include the α(1,2) fucosyltransferase (H transferase; Larsen et al., 1990A) and the α(1,3/1,4) fucosyltransferase (Weston et al., 1992). Of these, the H transferase is preferred and the human H transferase is particularly preferred. This transferase is responsible for synthesis of the H antigen which is the universal donor O-blood group antigen and utilizes the same acceptor substrates as the α(1,3) Gal transferase. Alternatively, although less preferred, sialyltransferases, e.g., the α(2,6) sialyltransferase (see Lowe, 1991), may be used in the practice of the invention.

Although the foregoing discussion and those that follow are phrased in terms of nucleic acid molecules encoding glycosyltransferases, the cells and methods of the invention more generally comprise nucleic acid molecules encoding any and all proteins that have glycosyltransferase activity, including, in particular, fucosyltransferase activity. Such proteins may be in the form of intact glycosyltranferases, but may also be in the form of proteins comprising active mutant glycosyltranferases such as those comprising active fragments of glycosyltranferases. See, for example, Kukowska, et al., 1991.

Vectors for expression of recombinant glycosyltransferases: In addition to the foregoing, the present invention provides vectors for the expression of recombinant glycosyltransferases in xenogeneic cells at levels effective to reduce the expression of Gal α(1,3) Gal epitopes by the xenogeneic cells into which the vectors have been introduced. Recombinant polynucleotides encoding glycosyltransferases that are appropriate for use in such vectors include those encoding the transferases discussed above. A particularly preferred polynucleotide is that encoding human H transferase, SEQ ID NO: 3.

The nucleic acid encoding the desired exogenous glycosyltransferase may be inserted into an appropriate parent expression vector, i.e., an expression vector that contains a site for inserting protein-encoding nucleic acid molecules, and also contains (in the appropriate orientation for expression) the necessary elements for the transcription and translation of an inserted protein-encoding sequence. Particularly preferred transcriptional and translational signals allow for expression of the desired glycosyltransferase in a wide variety of xenogeneic cell types.

A candidate parent expression vector can be tested for suitability for use in the practice of the present invention by the insertion of a nucleic acid fragment encoding the human H transferase into a site appropriate for expression in the parent expression vector, as described below in Example 1 for the APEX-1 vector, and testing cells containing the resulting expression vector for susceptibility to human complement-mediated damage as described below in Example 5.

The transcriptional and translational control sequences in mammalian expression vector systems to be used in genetically altering vertebrate cells may be provided by various sources, including viral sources. For example, commonly used promoters and enhancers known

to be generally operable in many mammalian cell types are derived from Polyoma virus, Adenovirus, Simian Virus 40 (SV40), and human cytomegalovirus immediate-early gene 1 promoter and enhancer (CMV). Cell-type specific promoters may also be used to express glycosyltransferases in particular cell types if desired.

A particularly preferred eukaryotic vector for the expression of glycosyltransferases in the methods and cells of the invention is pAPEX-1, SEQ ID NO:4 (see also copending U.S. patent application Serial No. 08/252,493, filed June 1, 1994, entitled "Porcine E-Selectin"). pAPEX-1 is a derivative of the vector pcDNAI/Amp (Invitrogen, San Diego CA) which was modified to increase protein expression in mammalian cells (see Example 1, below).

Whatever parent expression vector is used, transferase-encoding polynucleotide fragments will be subcloned into the parent vector, typically following digestion with appropriate restriction endonucleases. Fragments for such subcloning can be obtained by PCR amplification, restriction endonuclease digestion, and the like. These fragments and the parent vectors are assembled into a transferase expression vector using standard methods such as PCR fusion or enzymatic ligation (Sambrook, et al., 1989; Ausubel et al., 1992). Alternatively, nucleic acid molecules encoding the glycosyltransferases used in the methods and cells of the invention can be synthesized by chemical means (Talib, et al., 1991).

Expression vectors preferably also contain selectable markers, such as a beta lactamase antibiotic resistance gene for plasmid selection and propagation in microbial cells in the presence of an antibiotic such as ampicillin, and the neomycin gene for selection and propagation of stable mammalian transfectants, e.g., in the presence of the cytotoxic aminoglycoside G418.

Introduction of nucleic acid molecules into cells via transfection or transduction: As known in the art, introduction of nucleic acid molecules into cells can be accomplished by numerous methods, typically by transfection or transduction. Transfection methods include the addition of chemical carriers such as DEAE/dextran, calcium phosphate, or amphipathic lipids (in which case the procedure is generally referred to in the art as lipofection) to the nucleic acid molecules before or during the addition of those molecules to the cells to be transfected. Transfection methods also include mechanical means, such as electroporation, electric field mediated transfer (also referred to as Baekonization, see, for example, U.S. Pat. No. 4,849,355, entitled "Method Of Transferring Genes Into Cells" and U.S. Pat. No. 4,663,292, entitled "High-Voltage Biological Macromolecular Transfer And Cell Fusion System"), microinjection, and ballistic particle, "gene gun", mediated transfer.

The introduction of nucleic acid molecules into cells using engineered, replication incompetent viruses is referred to in the art as transduction. Transduction is a preferred method of nucleic acid molecule introduction into xenogeneic endothelial cells. The first step needed to use transduction methods in the practice of the present invention is incorporating the genetic sequence of the glycosyltransferase into a viral vector, e.g., a retroviral vector. Thereafter, the retroviral vectors are incorporated into retroviral vector particles using packaging cells.

The manipulation of retroviral nucleic acids to construct retroviral vectors and packaging cells is accomplished using techniques known in the art. See, for example, Ausubel, et al., 1992, Volume 1, Section III (units 9.10.1 - 9.14.3); Sambrook, et al., 1989; Miller, et al., 1989; Eglitis, et al., 1988; U.S. Patents Nos. 4,650,764, 4,861,719, 4,980,289, 5,122,767, and

5,124,263; as well as PCT Patent Publications Nos. WO 85/05629, WO 89/07150, WO 90/02797, WO 90/02806, WO 90/13641, WO 92/05266, WO 92/07943, WO 92/14829, and WO 93/14188.

In particular, retroviral vectors for use in the practice of the invention can be prepared and used as follows. First, a retroviral vector comprising a nucleic acid sequence encoding a glycosyltransferase is constructed from a parent retroviral vector. Examples of such parent retroviral vectors are found in, for example, Korman, et al., 1987; Morgenstern, et al., 1990; U.S. Patents Nos. 4,405,712, 4,980,289, and 5,112,767; and PCT Patent Publications Nos. WO 85/05629, WO 90/02797, and WO 92/07943. A preferred parent retroviral vector is the Moloney murine leukemia virus-derived expression vector pLXSN (Miller, et al., 1989).

The parent retroviral vector used in the practice of the present invention will be modified to include a glycosyltransferase encoding sequence and will be packaged into non-infectious (replication incompetent) transducing retroviral particles (virions) using an amphotropic packaging system, preferably one suitable for use in gene therapy applications.

Examples of useful packaging systems are found in, for example, Miller, et al., 1986; Markowitz, et al., 1988; Cosset, et al., 1990; U.S. Patents Nos. 4,650,764, 4,861,719, 4,980,289, 5,122,767, and 5,124,263, and PCT Patent Publications Nos. WO 85/05629, WO 89/07150, WO 90/02797, WO 90/02806, WO 90/13641, WO 92/05266, WO 92/07943, WO 92/14829, and WO 93/14188. A preferred packaging cell is the PA317 packaging cell line (ATCC CRL 9078).

The generation of "producer cells" is accomplished by introducing retroviral vectors into the packaging cells. The producer cells generated by the foregoing procedures are used to produce the retroviral vector

particles. This is accomplished by culturing of the cells in a suitable growth medium.

Preferably, the virions are harvested from the culture and administered to the target cells which are to be transduced. Examples of such target cells include isolated xenogeneic cells, cells of a xenogeneic organ or tissue, and other cells to be protected from antibody binding and complement attack, as well as xenogeneic progenitor cells, including stem cells such as embryonic or hematopoietic stem cells, which can be used to generate transgenic cells, tissues, or organs.

Alternatively, when practicable, virions are added to the target xenogeneic cells to be transduced by co-culture of the target cells with the producer cells. Suitable buffers and conditions for stable storage and subsequent use of the virions can be found in, for example, Ausubel, et al., 1992.

Cells, tissues, and organs: In general, any xenogeneic cell, tissue or organ may be utilized in the practice of the present invention. Preferred cells are of ungulate origin, and particularly preferred cells are of pig origin. The glycosyltransferase nucleic acid constructs of the invention can be used to engineer cultured cells of various types for subsequent use in transplantation. Examples of useful cell types include endothelial cells, fibroblastic and other skin cells, hepatic cells, neuronal and glial cells, pancreatic islet cells, hematopoietic cells, blood cells, lens cells, corneal cells, and stem cells.

Further, the glycosyltransferase nucleic acid constructs of the invention can be used to alter retroviral packaging cells or retroviral producer cells so that such cells exhibit a substantial reduction in the binding of naturally occurring preformed human antibodies or naturally occurring preformed Old World primate antibodies when compared to the binding of said antibodies to packaging or producer cells which have not

been so altered. In the discussion that follows, the expression "altered retroviral packaging/producer cells" is used to describe either or both of said altered packaging or producer cells. Such altered retroviral packaging/producer cells may be from any species that expresses Gal α(1,3) Gal epitopes, including cells of rodent or canine origin.

Among other applications, such altered retroviral packaging/producer cells may be used to provide gene therapy treatment in a patient in need of such treatment, e.g., for therapeutic control of neoplastic tumors. In this embodiment of the invention, altered retroviral producer cells producing a retroviral vector particle providing a therapeutic benefit are implanted into the patient. In the case of cancer therapy the implantation is preferably made into or adjacent to the tumor. In accordance with the invention, such altered producer cells are protected from HAR upon transplantation (implantation) into a human or Old World primate patient.

In addition, as disclosed in copending U.S. patent application Serial No. 08/278,639, entitled "Retroviral Transduction of Cells in the Presence of Complement", which is being filed concurrently herewith in the names of Russell P. Rother, Scott A. Rollins, William L. Fodor, and Stephen P. Squinto, the retroviral particles produced by the altered producer cells are protected from inactivation by complement in the body fluids of the patient. Other methods to protect retroviral vector particles from inactivation by complement in the body fluids of humans or Old World primates include those discussed in copending U.S. patent application Serial No. 08/278,550, entitled "Retroviral Transduction of Cells Using Soluble Complement Inhibitors", which is being filed concurrently herewith in the names of Russell P. Rother, Scott A. Rollins, James M. Mason, and Stephen P. Squinto, and in copending U.S. patent application Serial No. 08/278,630, entitled "Retroviral Vector Particles

Expressing Complement Inhibitor Activity", which is also being filed concurrently herewith in the names of James M. Mason and Stephen P. Squinto.

General discussions of packaging cells, retroviral vector particles and gene transfer using such particles can be found in various publications including PCT Patent Publication No. WO 92/07943, EPO Patent Publication No. 178,220, U.S. Patent No. 4,405,712, Gilboa, 1986; Mann, et al., 1983; Cone and Mulligan, 1984; Eglitis, et al., 1988; Miller, et al., 1989; Morgenstern and Land, 1990; Eglitis, 1991; Miller, 1992; Mulligan, 1993, and Ausubel, et al., 1992. The manipulation of retroviral nucleic acids to construct packaging vectors and packaging cells is discussed in, for example, Ausubel, et al., Volume 1, Section III (units 9.10.1 - 9.14.3), 1992; Sambrook, et al., 1989; Miller, et al., 1989; Eglitis, et al., 1988; U.S. Patents Nos. 4,650,764, 4,861,719, 4,980,289, 5,122,767, and 5,124,263; as well as PCT Patent Publications Nos. WO 85/05629, WO 89/07150, WO 90/02797, WO 90/02806, WO 90/13641, WO 92/05266, WO 92/07943, WO 92/14829, and WO 93/14188. To form packaging cells, packaging vectors are introduced into suitable host cells such as those found in, for example, Miller and Buttimore, Mol. Cell Biol., 6:2895-2902, 1986; Markowitz, et al., J. Virol., 62:1120-1124, 1988; Cosset, et al., J. Virol., 64:1070-1078, 1990; U.S. Patents Nos. 4,650,764, 4,861,719, 4,980,289, 5,122,767, and 5,124,263, and PCT Patent Publications Nos. WO 85/05629, WO 89/07150, WO 90/02797, WO 90/02806, WO 90/13641, WO 92/05266, WO 92/07943, WO 92/14829, and WO 93/14188. Once a packaging cell line has been established, producer cells are generated by introducing retroviral vectors into the packaging cells. Examples of such retroviral vectors are found in, for example, Korman, et al., 1987, Proc. Natl. Acad. Sci. USA, 84:2150-2154; Miller and Rosman, Biotechniques, 7:980-990, 1989; Morgenstern and Land, 1990; U.S. Patents Nos. 4,405,712, 4,980,289, and

5,112,767; and PCT Patent Publications Nos. WO 85/05629, WO 90/02797, and WO 92/07943. The retroviral vector includes a psi site and one or more exogenous nucleic acid sequences selected to perform a desired function, e.g., an experimental, diagnostic, or therapeutic function. These exogenous nucleic acid sequences are flanked by LTR sequences which function to direct high efficiency integration of the sequences into the genome of the ultimate target cell. (See also the discussion of transduction set forth above.)

Many applications of gene therapy using retroviral vector particles (RVVPs) are known and have been extensively reviewed (see, for example, Boggs, 1990; Kohn, et al., 1989; Lehn, 1990, Verma, 1990; Weatherall, 1991; and Felgner and Rhodes, 1991).

A variety of genes and DNA fragments can be incorporated into RVVPs for use in gene therapy. These DNA fragments and genes may encode RNA and/or protein molecules which render them useful as therapeutic agents. Protein encoding genes of use in gene therapy include those encoding various hormones, growth factors, enzymes, lymphokines, cytokines, receptors, and the like.

Among the genes which can be transferred are those encoding polypeptides that are absent, are produced in diminished quantities, or are produced in mutant form in individuals suffering from a genetic disease. Other genes of interest are those that encode proteins that, when expressed by a cell, can adapt the cell to grow under conditions where the unmodified cell would be unable to survive, or would become infected by a pathogen. Genes encoding proteins that have been engineered to circumvent a metabolic defect are also suitable for transfer into the cells of a patient. Such genes include the transmembrane form of CD59 discussed in copending U.S. patent application No. 08/205,720, filed March 3, 1994, entitled "Terminal Complement Inhibitor Fusion Genes and Proteins" and copending U.S. patent

application No. 08/206,189, filed March 3, 1994, entitled "Method for the Treatment of Paroxysmal Nocturnal Hemoglobinuria".

In addition to protein-encoding genes, RVVPs can be used to introduce nucleic acid sequences encoding medically useful RNA molecules into cells. Examples of such RNA molecules include anti-sense molecules and catalytic molecules, such as ribozymes.

In order to expedite rapid transduction by eliminating the need to wait for target cells to divide, and to allow transduction of cells that divide slowly or not at all, the use of RVVPs that can transduce non-dividing cells may be preferred. Such RVVPs are disclosed in copending U.S. patent applications Serial Nos. 08/181,335 and 08/182,612, both entitled "Retroviral Vector Particles for Transducing Non-Proliferating Cells" and both filed January 14, 1994. These patent applications also discuss specific procedures suitable for producing packaging vectors and retroviral vectors as well as the use of such vectors to produce packaging cells and producer cells, respectively.

Transgenic animals: Transgenic xenogeneic animals provide a preferred source of the cells, tissues, and organs of the invention. In accordance with certain aspects of the invention, the nucleic acid molecules of the invention are used to generate engineered transgenic animals, preferably ungulates (i.e., hooved animals such as pigs, cows, goats, sheep, and the like), that express the carbohydrate products of glycosyltransferases on the surfaces of their cells (e.g., endothelial cells) using techniques known in the art.

These techniques include, but are not limited to, microinjection (e.g., of pronuclei), electroporation of ova or zygotes, electric field mediated transfer (i.e., Baekonization, supra; see also Zhao and Wong, 1991), nuclear transplantation, and/or the stable transfection or transduction of embryonic stem cells derived from the

animal of choice. Electric field mediated transfer, i.e., Baekonization, is a preferred method of producing the transgenic animals of the invention.

A common element of these techniques involves the preparation of a transgene transcription unit. Such a unit comprises a DNA molecule which generally includes: 1) a promoter, 2) the nucleic acid sequence of interest, i.e., the sequence encoding a glycosyltransferase, and 3) a polyadenylation signal sequence. Other sequences, such as enhancer and intron sequences, can be included if desired. The unit can be conveniently prepared by isolating a restriction fragment of a plasmid vector which expresses the glycosyltransferase protein in, for example, mammalian cells. Preferably, the restriction fragment is free of sequences which direct replication in bacterial host cells since such sequences are known to have deleterious effects on embryo viability.

The most well known method for making transgenic animals is that used to produce transgenic mice by superovulation of a donor female, surgical removal of the egg, injection of the transgene transcription unit into the pro-nuclei of the embryo, and introduction of the transgenic embryo into the reproductive tract of a pseudopregnant host mother, usually of the same species. See Wagner, U.S. Patent No. 4,873,191, Brinster, et al., 1985, Hogan, et al., 1986, Robertson 1987, Pedersen, et al., 1990.

The use of this method to make transgenic ungulates is also widely practiced by those of skill in the art. As an example, transgenic swine are routinely produced by the microinjection of a transgene transcription unit into pig embryos. See, for example, PCT Publication No. WO92/11757. In brief, this procedure may, for example, be performed as follows.

First, the transgene transcription unit is gel isolated and extensively purified through, for example, an ELUTIP column (Schleicher & Schuell, Keene, NH),

dialyzed against pyrogen free injection buffer (10mM Tris, pH7.4 + 0.1mM EDTA in pyrogen free water) and used for embryo injection.

Embryos are recovered from the oviduct of a hormonally synchronized, ovulation induced sow, preferably at the pronuclear stage. They are placed into a 1.5 ml microfuge tube containing approximately 0.5 ml of embryo transfer media (phosphate buffered saline with 10% fetal calf serum). These are centrifuged for 12 minutes at 16,000 x g in a microcentrifuge. Embryos are removed from the microfuge tube with a drawn and polished Pasteur pipette and placed into a 35 mm petri dish for examination. If the cytoplasm is still opaque with lipid such that the pronuclei are not clearly visible, the embryos are centrifuged again for an additional 15 minutes.

Embryos to be microinjected are placed into a drop of media (approximately 100 μl) in the center of the lid of a 100 mm petri dish. Silicone oil is used to cover this drop and to fill the lid to prevent the medium from evaporating. The petri dish lid containing the embryos is set onto an inverted microscope equipped with both a heated stage (37.5-38°C) and Hoffman modulation contrast optics (200X final magnification).

A finely drawn and polished micropipette is used to stabilize the embryos while about 1-2 picoliters of injection buffer containing approximately 200-500 copies of the purified transgene transcription unit is delivered into the nucleus, preferably the male pronucleus, with another finely drawn and polished micropipette. Embryos surviving the microinjection process as judged by morphological observation are loaded into a polypropylene tube (2 mm ID) for transfer into the recipient pseudopregnant sow.

Offspring are tested for the presence of the transgene by isolating genomic DNA from tissue removed from the tail of each piglet and subjecting this genomic

DNA to nucleic acid hybridization analysis with transgene specific probes or PCR analysis with transgene specific primers.

Another commonly used technique for generating transgenic animals involves the genetic manipulation of embryonic stem cells (ES cells) as described in PCT Patent Publication No. WO 93/02188 and Robertson, 1987. In accordance with this technique, ES cells are grown as described in, for example, Robertson, 1987, and in U.S. Patent No. 5,166,065 to Williams et al., 1988. Genetic material is introduced into the embryonic stem cells by, for example, electroporation according, for example, to the method of McMahon, et al., 1990, or by transduction with a retroviral vector according, for example, to the method of Robertson, et al., 1986, or by any of the various techniques described by Lovell-Badge, 1987.

Chimeric animals are generated as described, for example, in Bradley, 1987. Briefly, genetically modified ES cells are introduced into blastocysts and the modified blastocysts are then implanted in pseudo-pregnant female animals. Chimeras are selected from the offspring, for example by the observation of mosaic coat coloration resulting from differences in the strain used to prepare the ES cells and the strain used to prepare the blastocysts, and are bred to produce non-chimeric transgenic animals.

Other methods for the production of transgenic animals are disclosed in U.S. Patent No. 5,032,407 to Wagner et al., and PCT Publication No. WO90/08832.

Among other applications, transgenic animals prepared in accordance with the invention are useful as model systems for testing the xenotransplantation of their engineered cells, tissues, and organs and as sources of engineered cells, tissues, and organs for xenotransplantation. The expression of functional glycosyltransferases by endothelial cells and/or other cell types in the tissues and organs of the transgenic

animals of the present invention will provide reduced susceptibility to hyperacute complement-mediated rejection following exposure of those cells, tissues, and organs to complement in human blood, plasma, serum, lymph, or the like, e.g., following xenotransplantation into humans or Old World primates. In accordance with the invention, reduced susceptibility to HAR is provided because naturally occurring preformed human or Old World primate antibodies have fewer binding sites on the transgenic cells of the invention.

Without intending to limit it in any manner, the present invention will be more fully described by the following examples.

Example 1

H Transferase

The human H transferase gene was cloned from cDNA prepared from Human Epidermoid Carcinoma cells (HEC cells, ATCC CRL 1555 #A-431) utilizing the Polymerase Chain Reaction (PCR). Cytoplasmic RNA was prepared from approximately $5X10^6$ cells, and first strand cDNA was synthesized from 5μg of RNA in a final volume of 100μl using the following reaction conditions: 10mM Tris-HCl pH8.3; 50mM KCl; 1.5mM $MgCl_2$; 500ng oligo$(dT)_{15}$ (Promega Corporation, Madison, Wisconsin); 10mM DTT; 0.25mM dNTPs (dG, dC, dA, dT); and 20U Avian Myeloblastosis Virus reverse transcriptase (Seikagaku of America, Inc., Rockville, Maryland) at 42°C for one hour.

PCR was performed following cDNA synthesis using 4μl of first strand cDNA reaction mixture as template and the following primers: a 34 base 5' primer homologous to the 5' untranslated region of the H transferase cDNA (SEQ ID NO:1; 5'-GGCCACGAAA AGCGGACTGT GGATCCGCCA CCTG-3'), where the underlined sequence represents a unique BamHI site; and a 38 base 3' primer homologous to the 3' UTR of the H transferase cDNA (SEQ ID NO:2; 5'-CAGGAACACC ACCAAGCTTC TCGAGAAGATGC CAGGCC-3'), in which the underlined sequence represents a unique XhoI site. PCR

reactions consisted of 35 cycles of 95°C - 1 minute, 52°C - 1 minute, and 72°C - 1.5 minutes. These 35 cycles were followed by a single ten minute extension at 72° C. An approximately 1300 bp band representing the PCR product was seen following agarose gel electrophoresis of an aliquot of the PCR reaction. This PCR product was cloned into a plasmid vector using the T/A cloning kit (Invitrogen, San Diego, CA). The pCRII plasmid vector included in this kit served as the recipient, and the resulting plasmid construct was amplified in E. coli and purified. Positive clones were identified by restriction endonuclease digestion and the insert was subsequently sequenced to confirm that the plasmid construct contained the human H transferase cDNA sequence shown in SEQ ID NO:3. An approximately 1200 bp BamHI-XhoI DNA fragment, encoding the full length H transferase enzyme, was gel isolated from the pCRII plasmid construct, electroeluted and subcloned into a BamHI-XhoI cut pAPEX-1 expression vector (see the following paragraph for a detailed description of this vector). Positive clones were identified by restriction mapping with BamHI-XhoI and StuI. Plasmid pAPEX1-HT, referred to hereinafter as pHT, was the result of these cloning and subcloning steps.

pAPEX-1 (SEQ. ID No:4) is a derivative of the vector pcDNAI/Amp (Invitrogen, San Diego CA) which was modified as follows to increase protein expression in mammalian cells. First, since the intron derived from the gene encoding the SV40 small-t antigen has been shown to decrease expression of upstream coding regions (Evans and Scarpulla, 1989), this intron was removed from pcDNAI/Amp by digestion with XbaI-HpaI, followed by treatment with the Klenow fragment of DNA polymerase and all four dNTPs. The resulting blunt ended 4.2 kb fragment was gel purified and self ligated to yield a closed circular plasmid. A 5'-untranslated region adenovirus/immunoglobulin hybrid intron was introduced into the plasmid by replacing a 0.5 kb NdeI-NotI fragment with the

corresponding 0.7 kb NdeI-NotI fragment from the vector pRc/CMV7SB (obtained from Dr. Joseph Goldstein, University of Texas Southwest Medical Center, Dallas, TX). Finally, the resulting CMV promoter expression cassette was shuttled as an NdeI-SfiI fragment into the vector pGEM-4Z (Promega, Madison WI) by ligation to an NdeI-SfiI fragment (containing pGEM-4Z) obtained from a pGEM based expression vector containing a CMV-promoter and an SV40 origin of replication (Davis et al., 1991).

Example 2

Transient Transfection of COS Cells with Porcine Galactose α(1,3) Galactosyltransferase and Human H Transferase

COS cells (ATCC # CRL 1650) were transiently transfected with CMV-based expression vectors. These vectors were pGT, containing an insert comprising a sequence (SEQ ID NO:5) encoding the pig α(1,3) Gal transferase oriented for CMV promoter-driven expression in the parent vector pCDNAI (Invitrogen, San Diego, CA), and pHT (described above), encoding the human H transferase. Clones containing pig α(1,3) Gal transferase cDNAs have been deposited with the Australian Government Analytical Laboratories, 1 Suakin Street, Pymble, N.S.W. 2073, Australia, and have been assigned the designations N94/9030 and N94/9029, respectively (see copending U.S. application Serial No. 08/214,580, supra). In a series of transfection experiments, the amount of pGT was kept constant at 3 μg/well and the amount of pHT was varied between 0 μg and 3 μg (Table 1). Transfection was carried out using the DEAE-dextran method (Seed and Aruffo, 1987).

COS cells maintained in DMEM with 10% FBS were seeded into 6-well tissue culture plates and were subsequently transfected with pHT and/or pGT. Transfected cells were examined for the expression of the Gal α(1,3) Gal epitope or the H epitope 48 hours after transfection. The cell surface expression of these two

epitopes was assessed using a fluoresceinated IB4 lectin, which binds specifically to the Gal α(1,3) Gal sugar structure, or by indirect immunofluorescence using a monoclonal antibody specific for the human H epitope (ASH-1952, obtained from the Austin Research Institute, Heidelberg, Victoria, Australia; see Devine, et al., 1990) and an immunopurified, fluorescein-conjugated Sheep anti-mouse IgG (Selenus Laboratories, Melbourne, Australia) as the secondary antibody. FIGS. 1-4 show the results of phase contrast (P) and fluorescence (F) microscopy of the cells obtained in these experiments.

In particular, FIGS. 1-4 are photomicrographs of African Green Monkey COS cells which have been fluorescently stained with ASH-1952 (FIGS. 2 and 3) or with IB4 (FIGS. 1 and 4). In each figure, the bottom panel shows all cells, as seen by phase contrast illumination, and the top panel shows only those cells specifically binding to the mAb or lectin as seen by ultraviolet illumination. The cells in FIG. 1 have been transfected with pGT; the cells in FIG. 2 have been transfected with pHT; and the cells in FIGS. 3 and 4 have been transfected with equal amounts of both vectors.

To assess the percentage of cells staining for the carbohydrate epitopes, 600-800 cells from each well were counted after staining. As shown in Table 1 and in FIGS. 1-2, most COS cells transiently transfected with the porcine Gal α(1,3) Gal transferase alone (3 μg) were positive for IB4 staining, and most COS cells transfected with the human H transferase alone (3 μg) were positive for anti-H staining. When equal amounts of the two expression plasmids were used (3 μg each), COS cells stained predominately for the H epitope (68% of cells), with only weak staining observed for the Gal α(1,3) Gal epitope (1.5% of cells). See Table 1 and FIGS. 3-4. In fact, even at a DNA ratio of 10:1 (3 μg pGT to 0.3 μg pHT), COS cells still stained predominately for the H

epitope (50.2 %) relative to the Gal α(1,3) Gal epitope (17.5%).

As a control, cotransfections were also done using expression vectors derived from a parent CMV-based expression vector (pCDM8; Seed and Aruffo, 1987) encoding either Ly-9 (Sandrin et al., 1992) or CD48 (Vaughan et al., 1991). Staining for the Ly-9 epitope was carried out using monoclonal antibody anti-Ly-9.2 (Sandrin et al., 1992). Staining for the CD48 epitope was carried out using an anti-CD48 monoclonal antibody (HuLy-m3; Vaughan et al., 1991). When equal amounts of the Ly-9 or CD48 expression vectors were cotransfected with either pGT or pHT, COS cells demonstrated intense staining for the appropriate carbohydrate epitope and for either CD48 or Ly-9, respectively. These results indicate that two different CMV-based expression vectors can function equally well when cotransfected into COS cells.

Example 3

Stable expression of H transferase in xenogeneic cells results in down-regulation of Gal α(1,3) Gal expression

A porcine kidney cell line (LLC-PK_1: ATCC# CRL 1392) was transfected with plasmid pHT (directing the expression of H transferase) and plasmid pSV2neo (directing the expression of the neomycin resistance gene encoding neomycin phosphotransferase) at a molar ratio of 20:1. Transfection was carried out by the calcium phosphate co-precipitation method and transfected cells were cultured in DMEM + 10% fetal bovine serum + G418 (500μg/ml, active). Stable neomycin resistant colonies were selected and expanded.

The cell surface expression of the H epitope was analyzed on G418 resistant colonies by indirect immunofluorescence performed with ASH-1952 (identified as "anti-H mAB" in FIG. 5) or with the H epitope specific lectin UEAI (EY Laboratories, Inc., San Mateo, CA.) directly conjugated to FITC. The Gal α(1,3) Gal cell surface epitope was visualized by staining control and

transfected cells with the FITC-conjugated lectin, IB4 (EY Laboratories, Inc., San Mateo, CA). As a control, transfected LLC-PK_1 cells were also stained with the anti-SLA class I (anti-pig major histocompatibility antigen class I) mAb, PT85A (VMRD, Inc., Pullman WA), as a positive control. Goat anti-mouse IgG antisera (monoclonal sera, Zymed Laboratories, South San Francisco, CA) directly conjugated to FITC was used to detect specific antibody binding to the cell surface by flow cytometry as shown in FIG. 5.

These data demonstrate that G418 resistant control LLC-PK_1 cells (clone PK1:neo #B6) normally express low levels of both the Gal α(1,3) Gal epitope and the H epitope (FIG. 5B) compared to staining with secondary antibody alone (FIG. 5D; 2° curve). However, cells transfected with the human H transferase vector (clone #A3) express high levels of the H epitope (FIG. 5A) and reduced levels of the Gal α(1,3) Gal epitope (FIG. 5A). Transfection of these cells with H transferase, however, did not alter the cell surface expression of the SLA class I gene product (FIG. 5C) relative to G418 resistant control cells (FIG. 5D).

Example 4

Stable expression of H transferase in xenogeneic cells results in significantly reduced binding of human IgG and IgM antibodies

Cell surface reactivity of human serum on the LLC-PK_1 transfectants was measure by incubation with 0% or 20% human whole serum followed by incubation with FITC conjugated goat anti-human antibodies specific for either human IgG or human IgM (Zymed Laboratories, South San Francisco, CA). Cell surface antibody binding was then measured by flow cytometry on a FACSort instrument (Becton-Dickinson Immunocytometry Systems, San Jose, CA). As shown in FIG. 6, LLC-PK_1 cells stably transfected with pHT demonstrate little to no reactivity to either human IgG (FIG. 6A) or IgM (FIG. 6B) relative to G418 resistant

control cells which demonstrate significant binding to human IgG (FIG. 6C) and IgM (FIG. 6D) present in 20% human serum. The binding of human IgG and IgM present in 20% human serum to H transferase-expressing LLC-PK_1 cells is similar to the binding observed with 0% whole human serum. These data together with the data presented in Example 3 indicate that expression of the H epitope on the surface of the LLC-PK_1 cells results in down-regulation of the expression of the Gal α(1,3) Gal epitope to such low levels that preformed naturally occurring human antibodies no longer bind to the cells.

Example 5

Stable expression of H transferase in xenogeneic cells results in significantly reduced sensitivity to human complement

The functional significance of recombinant H transferase expression by LLC-PK_1 cells was assessed by measuring the efflux of the trapped cytoplasmic indicator dye, Calcein AM (Molecular Probes, Inc.), from cells subjected to human complement-mediated damage by human serum. Transfected cells expressing the human H transferase and the neomycin resistance gene (clone #A3; see Examples 3 and 4 above) or the neomycin resistance gene alone (clone #C6; prepared in the same manner as clone B6 described above in Examples 3 and 4) were grown to confluence in 96-well plates. Cells were washed 2X with 200 μl of HBSS containing 1% (w/v) BSA (HBSS/BSA). Calcein AM was added (10mM final) and the plates were incubated at 37°C for 30 minutes. Subsequently, the cells were incubated at 37°C for 30 minutes in the presence of increasing concentrations of human whole serum.

Dye released from the cells was determined by the fluorescence in the supernatant. Total cell associated dye was determined from a 1% SDS cell lysate. The dye release was calculated as a percent of total, correcting for non-specific dye release and background fluorescence

measured for identically matched controls without the addition of serum. Fluorescence was measured using a Millipore Cytofluor 2350 fluorescence plate reader (490nm excitation, 530nm emission).

As shown in FIG. 7, LLC-PK_1 cells stably transfected with pHT (clone #A3; open triangles) were significantly less sensitive to the lytic activity of human complement relative to control LLC-PK_1 cells (clone #C6; closed circles) at all concentrations of human serum tested between 1% and 40%.

Throughout this application various publications, patents, and patent applications are referred to. The teachings and disclosures of these publications, patents, and patent applications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which the present invention pertains.

Although preferred and other embodiments of the invention have been described herein, further embodiments may be perceived by those skilled in the art without departing from the scope of the invention as defined by the following claims.

TABLE 1

pGT(μg)	pHT(μg)	IB4 % positive	ASH-1952 % positive
0.0	3.0	.01	69.0
3.0	3.0	1.5	68.0
3.0	1.5	4.5	70.3
3.0	1.0	4.6	65.8
3.0	0.3	17.5	50.2
3.0	0.15	43.4	34.0
3.0	0.03	61.5	28.9
3.0	0.0	68.4	0.0

pGT = porcine galactose α(1,3) galactosyltransferase cDNA subcloned into CMV-based expression plasmid pCDNAI (Invitrogen, Sand Diego, CA).

pHT = human H transferase cDNA subcloned into CMV-based expression plasmid pAPEX-1.

IB4 binds to the Gal α(1,3) Gal epitope.

ASH-1952 binds to the H epitope.

REFERENCES

Auchincloss, 1988. Transplantation 46, pp. 1.

Ausubel et al., 1992. Current Protocols in Mol Bio, Volume 1, Section III (units 9.10.1 - 9.14.3). John Wiley & Sons, New York.

Beyer and Hill, 1982, in Horowitz, (ed.). The Glycoconjugates Volume 3. Academic Press, NY, pp. 25-45.

Blanken and Van den Eijnden, 1985. J Biol Chem 260, pp. 12927-12934.

Boggs SS, Int. J. Cell Cloning, 8.80-96; 1990.

Bradley, 1987, in Robertson (ed). Teratocarcinomas and Embryonic Stem Cells a Practical Approach. IRL Press, Eynsham, Oxford, England.

Brent, 1991. Curr Opin Immunol 3, pp. 707.

Brinster et al., 1985. Proc Natl Acad Sci, USA 82, pp. 4438-4442.

Cairns et al., 1993A. Second International Congress on Xenotransplantation, pp. 56 (abstr).

Cairns et al., 1993B. Second International Congress on Xenotransplantation, pp. 156 (abstr).

Cone and Mulligan, Proc. Natl. Acad. Sci. USA, 81:6349-6353 1984.

Cooper et al., 1991, in Cooper et al., (eds). Xenotransplantation Heidelberg, Springer-Verlag, pp. 481-500.

Cosset et al., 1990. J Virol 64, pp. 1070-1078.

Dabkowski et al., 1993. Transplant Procs 25, pp. 2921.

Dalmasso et al., 1992. Am J Path 140, pp. 1157.

Davis et al., 1991. Science 253, pp. 59.

Devine, et al., 1990. J Tumor Marker Oncol 5(4), pp. 321-339.

Eglitis et al., 1988. Biotechniques 6, pp. 608-614.

Eglitis, 1991. Human Gene Therapy, 2:195-201.

Evans and Scarpulla, 1989. Gene 84, pp. 135.

Fabian et al., 1992. Transplant Procs 24, pp. 604.

Felgner PL, Rhodes G, Nature, 349:351-352; 1991.

Fournier et al., 1993. Second International Congress on Xenotransplantation, p 142 (abstr).

Galili et al., 1984. J Exp Med 160, pp. 1519.

Galili et al., 1985. J Exp Med 162, pp. 573.

Galili et al., 1987. Proc Natl Acad Sci, USA 84, pp. 1369.

Galili et al., 1988. J Biol Chem 263, pp. 17755-17762.

Galili, 1993. Immunology Today 14, pp. 480.

Geller et al., 1993. Transplantation 55, pp. 168.

Gilboa, Biotechniques, 4:504-512, 1986.

Good et al., 1992. Transplant Procs 24, pp. 558.

Hammer et al., 1992. Transplant Procs 24, pp. 590.

Hogan et al., 1986. Manipulating the Mouse Embryo: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Kohn DB, Anderson WF, Blaese RM, Cancer Investigation, 7:179-192;1989.

Koren et al., 1992. Transplant Proc 24, pp. 598.

Koren et al., 1993. Second International Congress on Xenotransplantation, pp. 178 (abstr).

Korman et al., 1987. Proc Natl Acad Sci, USA 84, pp. 2150-2154.

Kornfeld and Kornfeld, 1985. Ann Rev Biochem 54, pp. 631-664.

Kukowska et al., 1990. Genes Dev 4, pp. 11288-13039.

Larsen et al., 1990a. J Biol Chem 265, pp. 7055-7061.

Larsen et al., 1990b. Proc Natl Acad Sci USA 87, pp. 6674-6678.

Lehn PM, Bone Marrow Transplantation, 5:287-293;1990.

Lovell-Badge, 1987, in Robertson (ed). Teratocarcinomas and Embryonic Stem Cells a Practical Approach. IRL Press, Eynsham, Oxford, England.

Lowe, 1991. Sem Cell Biol 2, pp. 289-307.

Mann, et al., Cell, 33:153-159, 1983.

Markowitz et al., 1988. J Virol 62, pp. 1120-1124.

McMahon et al., 1990. Cell 62, pp. 1073-1085.

Mejia-Laguna et al., 1972. Am J Path 69, pp. 71.

Miller et al., 1986. Mol Cell Bio 6, pp. 2895-2902.

Miller et al., 1989. Biotechniques 7, pp. 981-990.

Miller, 1992, Nature, 357:455-460.

Morgenstern et al., 1990. Nucleic Acids Res 18, pp. 3587-3596.

Muler-Eberhard, 1988. Ann Rev Biochem 57, pp. 321.

Mulligan, 1993, Science, 260:926-932.

Najarian, 1992. Transplant Proc 24, pp. 733.

Niekrasz et al., 1992. Transplant Procs 24, pp. 625-626.

Pedersen et al., 1990. Transgenic Techniques in Mice - A Video Guide. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Platt and Bach, 1991. Curr Opin Immunol 3, pp. 735.

Platt et al., 1990. Immunology Today 11, pp. 450.

Platt et al., 1991. Transplantation 52, pp. 214.

Robertson et al., 1986. Nature 323, pp. 445-448.

Robertson, 1987, in Robertson (ed). Teratocarcinomas and Embryonic Stem Cells a Practical Approach. IRL Press, Eynsham, Oxford, England.

Sambrook et al., 1989. Molecular Cloning: A Laboratory Manual, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sandrin et al., 1992. J Immunol 149, pp. 1636-1641.

Sandrin et al., 1993A. Proc Natl Acad Sci, USA 90, pp. 11391-11395.

Sandrin et al., 1993B. Transplant Proc 25, pp. 2917.

Seed and Aruffo, 1987. Proc Natl Acad Sci, USA 84, pp. 3365.

Somervile and d'Apice, 1993. Kidney Intl 44, pp. 112.

Stanley, 1992. Glycobiol 2(2), pp. 99-107.

Talib et al., 1991. Gene 98, pp. 289-293.

Thall and Galili, 1990. Biochemistry 29, pp. 3959.

Tusso et al., 1992. Transplant Procs 24, pp. 596.

Tusso et al., 1993. Transplantation 55, pp. 1375.

Vaughan et al., 1991. Immunogenetics 33, pp. 113-117.

Verma IM, Scientific American, pp. 68-84;1990.

Weatherall DJ, Nature, 349:275-276; 1991.

Weston et al., 1992. J Biol Chem 267, pp. 4152-4160.

Williams et al., 1988. Immunogenetics 27, pp. 265-272.

Yamakawa and Nagai, 1978. Trends Biol Sci 3, pp. 128-131.

Zehr et al., 1994. Transplantation 57, pp. 900.

Zhao and Wong, 1991. J Cell Biol 115, pp. 83a.

Zhao et al., 1993. Second International Congress on Xenotransplantation, pp. 138 (abstr).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Sandrin, Mauro S.
Fodor, William L.
Rother, Russell P.
Squinto, Stephen P.
McKenzie, Ian F. C.

(ii) TITLE OF INVENTION: Methods for Reducing Hyperacute Rejection of Xenografts

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Maurice M. Klee

(B) STREET: 1951 Burr Street

(C) CITY: Fairfield

(D) STATE: Connecticut

(E) COUNTRY: USA

(F) ZIP: 06430

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 inch, 750 Kb storage

(B) COMPUTER: Dell 486/50

(C) OPERATING SYSTEM: DOS 6.2

(D) SOFTWARE: WordPerfect 6.0

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/260,201

(B) FILING DATE: June 15, 1994

(C) CLASSIFICATION:

(D) APPLICATION NUMBER: 08/278,282

(E) FILING DATE: July 21, 1994

(F) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Klee, Maurice M.

(B) REGISTRATION NUMBER: 30,399

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(ix) TELECOMMUNICATION INFORMATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 bases

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid

(A) DESCRIPTION: Oligonucleotide primer

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCCACGAAA AGCGGACTGT GGATCCGCCA CCTG 34

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 bases

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Other nucleic acid

(A) DESCRIPTION: Oligonucleotide primer

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CAGGAACACC ACCAAGCTTC TCGAGAAGAT GCCAGGCC 38

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1174 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA to mRNA

(A) DESCRIPTION: Human H-transferase

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Larsen, R.D.
Ernst, L.K.
Nair, R.P.
Lowe, J.B.

(B) TITLE: Molecular cloning, sequence, and expression of a human GDP-L-fucose: -D-galactoside 2-alpha-L-fucosyltransferase cDNA that can form the H blood group antigen.

(C) JOURNAL: Proceedings of the National Academy of Sciences, USA

(D) VOLUME: 87

(F) PAGES: 6674-6678

(G) DATE: SEP-1990

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAAGCAGCTC GGCC 14

ATG TGG CTC CGG AGC CAT CGT CAG CTC TGC CTG GCC TTC CTG 56
Met Trp Leu Arg Ser His Arg Gln Leu Cys Leu Ala Phe Leu
1 5 10

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CTA GTC TGT GTC CTC TCT GTA ATC TTC TTC CTC CAT ATC CAT        98
Leu Val Cys Val Leu Ser Val Ile Phe Phe Leu His Ile His
 15                  20                  25

CAA GAC AGC TTT CCA CAT GGC CTA GGC CTG TCG ATC CTG TGT       140
Gln Asp Ser Phe Pro His Gly Leu Gly Leu Ser Ile Leu Cys
     30                  35                  40

CCA GAC CGC CGC CTG GTG ACA CCC CCA GTG GCC ATC TTC TGC       182
Pro Asp Arg Arg Leu Val Thr Pro Pro Val Ala Ile Phe Cys
         45                  50                  55

CTG CCG GGT ACT GCG ATG GGC CCC AAC GCC TCC TCT TCC TGT       224
Leu Pro Gly Thr Ala Met Gly Pro Asn Ala Ser Ser Ser Cys
             60                  65                  70

CCC CAG CAC CCT GCT TCC CTC TCC GGC ACC TGG ACT GTC TAC       266
Pro Gln His Pro Ala Ser Leu Ser Gly Thr Trp Thr Val Tyr
                 75                  80

CCC AAT GGC CGG TTT GGT AAT CAG ATG GGA CAG TAT GCC ACG       308
Pro Asn Gly Arg Phe Gly Asn Gln Met Gly Gln Tyr Ala Thr
 85                  90                  95

CTG CTG GCT CTG GCC CAG CTC AAC GGC CGC CGG GCC TTT ATC       350
Leu Leu Ala Leu Ala Gln Leu Asn Gly Arg Arg Ala Phe Ile
     100                 105                 110

CTG CCT GCC ATG CAT GCC GCC CTG GCC CCG GTA TTC CGC ATC       392
Leu Pro Ala Met His Ala Ala Leu Ala Pro Val Phe Arg Ile
         115                 120                 125

ACC CTG CCC GTG CTG GCC CCA GAA GTG GAC AGC CGC ACG CCG       434
Thr Leu Pro Val Leu Ala Pro Glu Val Asp Ser Arg Thr Pro
             130                 135                 140

TGG CGG GAG CTG CAG CTT CAC GAC TGG ATG TCG GAG GAG TAC       476
Trp Arg Glu Leu Gln Leu His Asp Trp Met Ser Glu Glu Tyr
                 145                 150

GCG GAC TTG AGA GAT CCT TTC CTG AAG CTC TCT GGC TTC CCC       518
Ala Asp Leu Arg Asp Pro Phe Leu Lys Leu Ser Gly Phe Pro
155                 160                 165

TGC TCT TGG ACT TTC TTC CAC CAT CTC CGG GAA CAG ATC CGC       560
Cys Ser Trp Thr Phe Phe His His Leu Arg Glu Gln Ile Arg
    170                 175                 180

AGA GAG TTC ACC CTG CAC GAC CAC CTT CGG GAA GAG GCG CAG       602
Arg Glu Phe Thr Leu His Asp His Leu Arg Glu Glu Ala Gln
        185                 190                 195

AGT GTG CTG GGT CAG CTC CGC CTG GGC CGC ACA GGG GAC CGC       644
Ser Val Leu Gly Gln Leu Arg Leu Gly Arg Thr Gly Asp Arg
            200                 205                 210
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CCG CGC ACC TTT GTC GGC GTC CAC GTG CGC CGT GGG GAC TAT          686
Pro Arg Thr Phe Val Gly Val His Val Arg Arg Gly Asp Tyr
                215                 220

CTG CAG GTT ATG CCT CAG CGC TGG AAG GGT GTG GTG GGC GAC          728
Leu Gln Val Met Pro Gln Arg Trp Lys Gly Val Val Gly Asp
225                 230                 235

AGC GCC TAC CTC CGG CAG GCC ATG GAC TGG TTC CGG GCA CGG          770
Ser Ala Tyr Leu Arg Gln Ala Met Asp Trp Phe Arg Ala Arg
    240                 245                 250

CAC GAA GCC CCC GTT TTC GTG GTC ACC AGC AAC GGC ATG GAG          812
His Glu Ala Pro Val Phe Val Val Thr Ser Asn Gly Met Glu
        255                 260                 265

TGG TGT AAA GAA AAC ATC GAC ACC TCC CAG GGC GAT GTG ACG          854
Trp Cys Lys Glu Asn Ile Asp Thr Ser Gln Gly Asp Val Thr
            270                 275                 280

TTT GCT GGC GAT GGA CAG GAG GCT ACA CCG TGG AAA GAC TTT          896
Phe Ala Gly Asp Gly Gln Glu Ala Thr Pro Trp Lys Asp Phe
                285                 290

GCC CTG CTC ACA CAG TGC AAC CAC ACC ATT ATG ACC ATT GGC          938
Ala Leu Leu Thr Gln Cys Asn His Thr Ile Met Thr Ile Gly
295                 300                 305

ACC TTC GGC TTC TGG GCT GCC TAC CTG GCT GGC GGA GAC ACT          980
Thr Phe Gly Phe Trp Ala Ala Tyr Leu Ala Gly Gly Asp Thr
    310                 315                 320

GTC TAC CTG GCC AAC TTC ACC CTG CCA GAC TCT GAG TTC CTG         1022
Val Tyr Leu Ala Asn Phe Thr Leu Pro Asp Ser Glu Phe Leu
        325                 330                 335

AAG ATC TTT AAG CCG GAG GCG GCC TTC CTG CCC GAG TGG GTG         1064
Lys Ile Phe Lys Pro Glu Ala Ala Phe Leu Pro Glu Trp Val
            340                 345                 350

GGC ATT AAT GCA GAC TTG TCT CCA CTC TGG ACA TTG GCT AAG         1106
Gly Ile Asn Ala Asp Leu Ser Pro Leu Trp Thr Leu Ala Lys
                355                 360

CCT TGAGAGCCAG GGAGACTTTC TGAAGTAGCC TGATCTTTCT                 1149
Pro
365

AGAGCCAGCA GTACGTGGCT TCAGA                                    1174
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4059 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Circular

(ii) MOLECULE TYPE: Other nucleic acid

(A) DESCRIPTION: Apex-1 Eukaryotic (CMV) Expression Vector

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ACGCGTTGAC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG 50

GTCATTAGTT CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG 100

TAAATGGCCC CGCCTGGCTG ACCGCCCAAC GACCCCCGCC CATTGACGTC 150

AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC 200

GTCAATGGGT GGACTATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA 250

GTGTATCATA TGCCAAGTAC GCCCCCTATT GACGTCAATG ACGGTAAATG 300

GCCCGCCTGG CATTATGCCC AGTACATGAC CTTATGGGAC TTTCCTACTT 350

GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT GATGCGGTTT 400

TGGCAGTACA TCAATGGGCG TGGATAGCGG TTTGACTCAC GGGGATTTCC 450

AAGTCTCCAC CCCATTGACG TCAATGGGAG TTTGTTTTGG CACCAAAATC 500

AACGGGACTT TCCAAAATGT CGTAACAACT CCGCCCCATT GACGCAAATG 550

GGCGGTAGGC GTGTACGGTG GGAGGTCTAT ATAAGCAGAG CTCGTTTAGT 600

GAACCGTCAG AATTCTGTTG GGCTCGCGGT TGATTACAAA CTCTTCGCGG 650

TCTTTCCAGT ACTCTTGGAT CGGAAACCCG TCGGCCTCCG AACGGTACTC 700

CGCCACCGAG GGACCTGAGC GAGTCCGCAT CGACCGGATC GGAAAACCTC 750

TCGACTGTTG GGGTGAGTAC TCCCTCTCAA AAGCGGGCAT GACTTCTGCG 800

CTAAGATTGT CAGTTTCCAA AAACGAGGAG GATTTGATAT TCACCTGGCC 850

CGCGGTGATG CCTTTGAGGG TGGCCGCGTC CATCTGGTCA GAAAAGACAA 900

TCTTTTTGTT GTCAAGCTTG AGGTGTGGCA GGCTTGAGAT CTGGCCATAC 950

ACTTGAGTGA CAATGACATC CACTTTGCCT TTCTCTCCAC AGGTGTCCAC 1000

TCCCAGGTCC AACTGCAGGT CGACCGGCTT GGTACCGAGC TCGGATCCAC 1050

TAGTAACGGC CGCCAGTGTG CTGGAATTCT GCAGATATCC ATCACACTGG 1100

CGGCCGCTCG AGCATGCATC TAGAACTTGT TTATTGCAGC TTATAATGGT 1150

TACAAATAAA GCAATAGCAT CACAAATTTC ACAAATAAAG CATTTTTTTC 1200

ACTGCATTCT AGTTGTGGTT TGTCCAAACT CATCAATGTA TCTTATCATG 1250

TCTGGATCGA TCCCGCCATG GTATCAACGC CATATTTCTA TTTACAGTAG 1300

GGACCTCTTC GTTGTGTAGG TACCGCTGTA TTCCTAGGGA AATAGTAGAG 1350

GCACCTTGAA CTGTCTGCAT CAGCCATATA GCCCCCGCTG TTCGACTTAC 1400

AAACACAGGC ACAGTACTGA CAAACCCATA CACCTCCTCT GAAATACCCA 1450

TAGTTGCTAG GGCTGTCTCC GAACTCATTA CACCCTCCAA AGTCAGAGCT 1500

GTAATTTCGC CATCAAGGGC AGCGAGGGCT TCTCCAGATA AAATAGCTTC 1550

TGCCGAGAGT CCCGTAAGGG TAGACACTTC AGCTAATCCC TCGATGAGGT 1600

CTACTAGAAT AGTCAGTGCG GCTCCCATTT TGAAAATTCA CTTACTTGAT 1650

CAGCTTCAGA AGATGGCGGA GGGCCTCCAA CACAGTAATT TTCCTCCCGA 1700

CTCTTAAAAT AGAAAATGTC AAGTCAGTTA AGCAGGAAGT GGACTAACTG 1750

ACGCAGCTGG CCGTGCGACA TCCTCTTTTA ATTAGTTGCT AGGCAACGCC 1800

CTCCAGAGGG CGTGTGGTTT TGCAAGAGGA AGCAAAAGCC TCTCCACCCA 1850

GGCCTAGAAT GTTTCCACCC AATCATTACT ATGACAACAG CTGTTTTTTT 1900

TAGTATTAAG CAGAGGCCGG GGACCCCTGG GCCCGCTTAC TCTGGAGAAA 1950

AAGAAGAGAG GCATTGTAGA GGCTTCCAGA GGCAACTTGT CAAAACAGGA 2000

CTGCTTCTAT TTCTGTCACA CTGTCTGGCC CTGTCACAAG GTCCAGCACC 2050

TCCATACCCC CTTTAATAAG CAGTTTGGGA ACGGGTGCGG GTCTTACTCC 2100

GCCCATCCCG CCCCTAACTC CGCCCAGTTC CGCCCATTCT CCGCCCCATG 2150

GCTGACTAAT TTTTTTTATT TATGCAGAGG CCGAGGCCGC CTCGGCCTCT 2200

GAGCTATTCC AGAAGTAGTG AGGAGGCTTT TTTGGAGGCC TAGGCTTTTG 2250

CAAAAAGGAG CTCCCAGCAA AAGGCCAGGA ACCGTAAAAA GGCCGCGTTG 2300

CTGGCGTTTT TCCATAGGCT CCGCCCCCCT GACGAGCATC ACAAAAATCG 2350

ACGCTCAAGT CAGAGGTGGC GAAACCCGAC AGGACTATAA AGATACCAGG 2400

CGTTTCCCCC TGGAAGCTCC CTCGTGCGCT CTCCTGTTCC GACCCTGCCG 2450

CTTACCGGAT ACCTGTCCGC CTTTCTCCCT TCGGGAAGCG TGGCGCTTTC 2500

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AGCTGGGCTG TGTGCACGAA CCCCCCGTTC AGCCCGACCG CTGCGCCTTA 2600

TCCGGTAACT ATCGTCTTGA GTCCAACCCG GTAAGACACG ACTTATCGCC 2650

ACTGGCAGCA GCCACTGGTA ACAGGATTAG CAGAGCGAGG TATGTAGGCG 2700

GTGCTACAGA GTTCTTGAAG TGGTGGCCTA ACTACGGCTA CACTAGAAGG 2750

ACAGTATTTG GTATCTGCGC TCTGCTGAAG CCAGTTACCT TCGGAAAAAG 2800

AGTTGGTAGC TCTTGATCCG GCAAACAAAC CACCGCTGGT AGCGGTGGTT 2850

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GATCCTTTGA TCTTTTCTAC GGGGTCTGAC GCTCAGTGGA ACGAAAACTC 2950

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TAAACTTGGT CTGACAGTTA CCAATGCTTA ATCAGTGAGG CACCTATCTC 3100

AGCGATCTGT CTATTTCGTT CATCCATAGT TGCCTGACTC CCCGTCGTGT 3150

AGATAACTAC GATACGGGAG GGCTTACCAT CTGGCCCCAG TGCTGCAATG 3200

ATACCGCGAG ACCCACGCTC ACCGGCTCCA GATTTATCAG CAATAAACCA 3250

GCCAGCCGGA AGGGCCGAGC GCAGAAGTGG TCCTGCAACT TTATCCGCCT 3300

CCATCCAGTC TATTAATTGT TGCCGGGAAG CTAGAGTAAG TAGTTCGCCA 3350

GTTAATAGTT TGCGCAACGT TGTTGCCATT GCTACAGGCA TCGTGGTGTC 3400

ACGCTCGTCG TTTGGTATGG CTTCATTCAG CTCCGGTTCC CAACGATCAA 3450

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GGTTATGGCA GCACTGCATA ATTCTCTTAC TGTCATGCCA TCCGTAAGAT 3600

GCTTTTCTGT GACTGGTGAG TACTCAACCA AGTCATTCTG AGAATAGTGT 3650

ATGCGGCGAC CGAGTTGCTC TTGCCCGGCG TCAATACGGG ATAATACCGC 3700

GCCACATAGC AGAACTTTAA AAGTGCTCAT CATTGGAAAA CGTTCTTCGG 3750

GGCGAAAACT CTCAAGGATC TTACCGCTGT TGAGATCCAG TTCGATGTAA 3800

CCCACTCGTG CACCCAACTG ATCTTCAGCA TCTTTTACTT TCACCAGCGT 3850

TTCTGGGTGA GCAAAAACAG GAAGGCAAAA TGCCGCAAAA AAGGGAATAA 3900

GGGCGACACG GAAATGTTGA ATACTCATAC TCTTCCTTTT TCAATATTAT 3950

TGAAGCATTT ATCAGGGTTA TTGTCTCATG AGCGGATACA TATTTGAATG 4000

TATTTAGAAA AATAAACAAA TAGGGGTTCC GCGCACATTT CCCCGAAAAG 4050

TGCCACCTG 4059

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1423 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Double

(D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: cDNA to mRNA

(A) DESCRIPTION: galactosyl transferase, full coding sequence

(iii) HYPOTHETICAL: No

(iv) ANTI-SENSE: No

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Sus scrofa

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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CGGGGGCCAT CCCCGAGCGC ACCCAGCTTC TGCCGATCAG GAGAAAATA                49

ATG AAT GTC AAA GGA AGA GTG GTT CTG TCA ATG CTG CTT GTC               91
Met Asn Val Lys Gly Arg Val Val Leu Ser Met Leu Leu Val
                  5                  10

TCA ACT GTA ATG GTT GTG TTT TGG GAA TAC ATC AAC AGA AAC              133
Ser Thr Val Met Val Val Phe Trp Glu Tyr Ile Asn Arg Asn
 15                  20                  25

CCA GAA GTT GGC AGC AGT GCT CAG AGG GGC TGG TGG TTT CCG              175
Pro Glu Val Gly Ser Ser Ala Gln Arg Gly Trp Trp Phe Pro
     30                  35                  40

AGC TGG TTT AAC AAT GGG ACT CAC AGT TAC CAC GAA GAA GAA              217
Ser Trp Phe Asn Asn Gly Thr His Ser Tyr His Glu Glu Glu
         45                  50                  55

GAC GCT ATA GGC AAC GAA AAG GAA CAA AGA AAA GAA GAC AAC              259
Asp Ala Ile Gly Asn Glu Lys Glu Gln Arg Lys Glu Asp Asn
             60                  65                  70
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AGA GGA GAG CTT CCG CTA GTG GAC TGG TTT AAT CCT GAG AAA      301
Arg Gly Glu Leu Pro Leu Val Asp Trp Phe Asn Pro Glu Lys
                75                  80

CGC CCA GAG GTC GTG ACC ATA ACC AGA TGG AAG GCT CCA GTG      343
Arg Pro Glu Val Val Thr Ile Thr Arg Trp Lys Ala Pro Val
 85                  90                  95

GTA TGG GAA GGC ACT TAC AAC AGA GCC GTC TTA GAT AAT TAT      385
Val Trp Glu Gly Thr Tyr Asn Arg Ala Val Leu Asp Asn Tyr
    100                 105                 110

TAT GCC AAA CAG AAA ATT ACC GTG GGC TTG ACG GTT TTT GCT      427
Tyr Ala Lys Gln Lys Ile Thr Val Gly Leu Thr Val Phe Ala
        115                 120                 125

GTC GGA AGA TAC ATT GAG CAT TAC TTG GAG GAG TTC TTA ATA      469
Val Gly Arg Tyr Ile Glu His Tyr Leu Glu Glu Phe Leu Ile
            130                 135                 140

TCT GCA AAT ACA TAC TTC ATG GTT GGC CAC AAA GTC ATC TTT      511
Ser Ala Asn Thr Tyr Phe Met Val Gly His Lys Val Ile Phe
                145                 150

TAC ATC ATG GTG GAT GAT ATC TCC AGG ATG CCT TTG ATA GAG      553
Tyr Ile Met Val Asp Asp Ile Ser Arg Met Pro Leu Ile Glu
155                 160                 165

CTG GGT CCT CTG CGT TCC TTT AAA GTG TTT GAG ATC AAG TCC      595
Leu Gly Pro Leu Arg Ser Phe Lys Val Phe Glu Ile Lys Ser
    170                 175                 180

GAG AAG AGG TGG CAA GAC ATC AGC ATG ATG CGC ATG AAG ACC      637
Glu Lys Arg Trp Gln Asp Ile Ser Met Met Arg Met Lys Thr
        185                 190                 195

ATC GGG GAG CAC ATC CTG GCC CAC ATC CAG CAC GAG GTG GAC      679
Ile Gly Glu His Ile Leu Ala His Ile Gln His Glu Val Asp
            200                 205                 210

TTC CTC TTC TGC ATT GAC GTG GAT CAG GTC TTC CAA AAC AAC      721
Phe Leu Phe Cys Ile Asp Val Asp Gln Val Phe Gln Asn Asn
                215                 220

TTT GGG GTG GAG ACC CTG GGC CAG TCG GTG GCT CAG CTA CAG      763
Phe Gly Val Glu Thr Leu Gly Gln Ser Val Ala Gln Leu Gln
225                 230                 235

GCC TGG TGG TAC AAG GCA CAT CCT GAC GAG TTC ACC TAC GAG      805
Ala Trp Trp Tyr Lys Ala His Pro Asp Glu Phe Thr Tyr Glu
    240                 245                 250

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Arg Arg Lys Glu Ser Ala Ala Tyr Ile Pro Phe Gly Gln Gly
        255                 260                 265
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GAT TTT TAT TAC CAC GCA GCC ATT TTT GGG GGA ACA CCC ACT      889
Asp Phe Tyr Tyr His Ala Ala Ile Phe Gly Gly Thr Pro Thr
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CAG GTT CTA AAC ATC ACT CAG GAG TGC TTC AAG GGA ATC CTC      931
Gln Val Leu Asn Ile Thr Gln Glu Cys Phe Lys Gly Ile Leu
                285                 290

CAG GAC AAG GAA AAT GAC ATA GAA GCC GAG TGG CAT GAT GAA      973
Gln Asp Lys Glu Asn Asp Ile Glu Ala Glu Trp His Asp Glu
295                 300                 305

AGC CAT CTA AAC AAG TAT TTC CTT CTC AAC AAA CCC ACT AAA     1015
Ser His Leu Asn Lys Tyr Phe Leu Leu Asn Lys Pro Thr Lys
    310                 315                 320

ATC TTA TCC CCA GAA TAC TGC TGG GAT TAT CAT ATA GGC ATG     1057
Ile Leu Ser Pro Glu Tyr Cys Trp Asp Tyr His Ile Gly Met
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TCT GTG GAT ATT AGG ATT GTC AAG ATA GCT TGG CAG AAA AAA     1099
Ser Val Asp Ile Arg Ile Val Lys Ile Ala Trp Gln Lys Lys
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Glu Tyr Asn Leu Val Arg Asn Asn Ile
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TATCTGCGGA ATTCCAGCTG AGCGCCGGTC GCTACCATTA CCAGTTGGTC      1386

TGGTGTCGAC GACTCCTGGA GCCCGTCAGT ATCGGCG                    1423
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What is claimed is:

1. A method for reducing rejection of a xenogeneic cell following transplantation into a human or an Old World primate comprising:

(a) producing a genetically altered cell by introducing an expression vector comprising a nucleic acid sequence encoding a protein having fucosyltransferase activity into a recipient cell, the introduction of said expression vector causing a substantial reduction in the binding of naturally occurring preformed human antibodies or naturally occurring preformed Old World primate antibodies to the genetically altered cell when compared to the binding of said antibodies to the recipient cell; and

(b) transplanting said genetically altered cell or a cell derived from said cell into a human or an Old World primate.

2. The method of Claim 1 wherein the genetically altered cell is an ungulate cell.

3. The method of Claim 1 wherein the genetically altered cell is a retroviral producer cell.

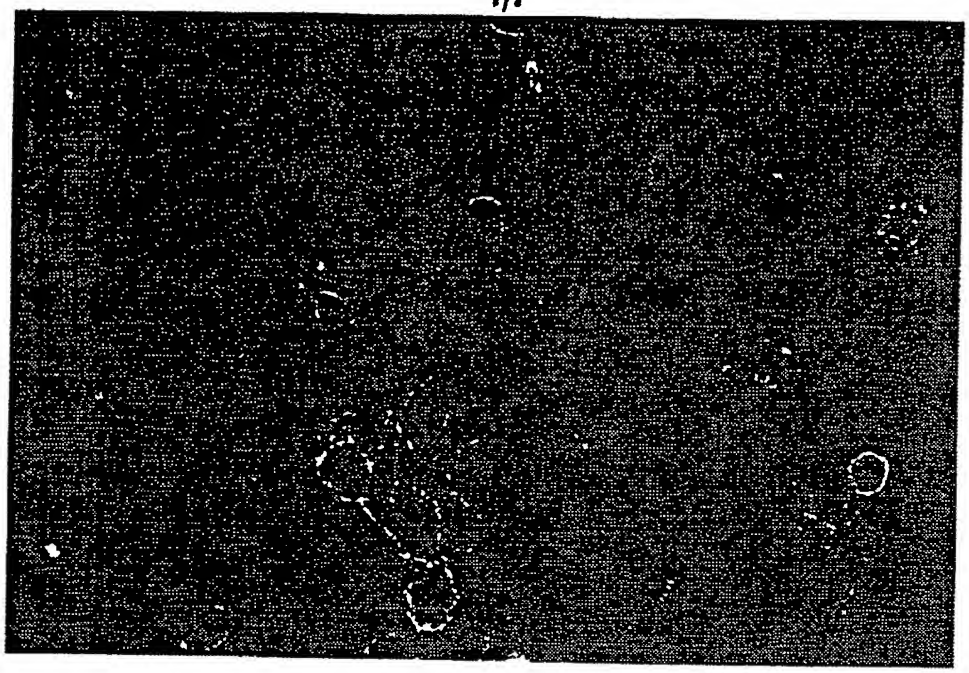
4. An ungulate cell which has been genetically altered by the introduction of an expression vector comprising a nucleic acid sequence encoding a protein having fucosyltransferase activity into a recipient ungulate cell, the introduction of said expression vector causing a substantial reduction in the binding of naturally occurring preformed human antibodies or naturally occurring preformed Old World primate antibodies to said genetically altered ungulate cell when compared to the binding of said antibodies to the recipient ungulate cell.

5. An ungulate cell, tissue, or organ derived from the genetically altered ungulate cell of Claim 4.

6. A retroviral packaging cell which has been genetically altered by the introduction of an expression vector comprising a nucleic acid sequence encoding a

protein having fucosyltransferase activity into a recipient cell from which the genetically altered retroviral packaging cell is derived, the introduction of said expression vector causing a substantial reduction in the binding of naturally occurring preformed human antibodies or naturally occurring preformed Old World primate antibodies to said genetically altered retroviral packaging cell when compared to the binding of said antibodies to the recipient cell from which the genetically altered retroviral packaging cell is derived.

7. A retroviral producer cell which has been genetically altered by the introduction of an expression vector comprising a nucleic acid sequence encoding a protein having fucosyltransferase activity into a recipient cell from which the genetically altered retroviral producer cell is derived, the introduction of said expression vector causing a substantial reduction in the binding of naturally occurring preformed human antibodies or naturally occurring preformed Old World primate antibodies to said genetically altered retroviral producer cell when compared to the binding of said antibodies to the recipient cell from which the genetically altered retroviral producer cell is derived.



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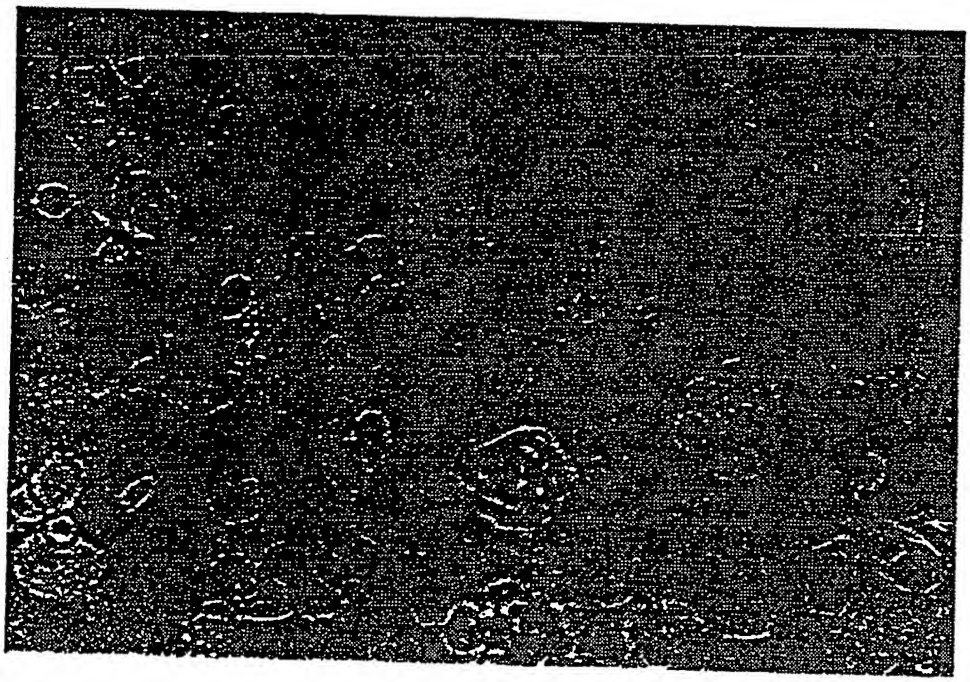

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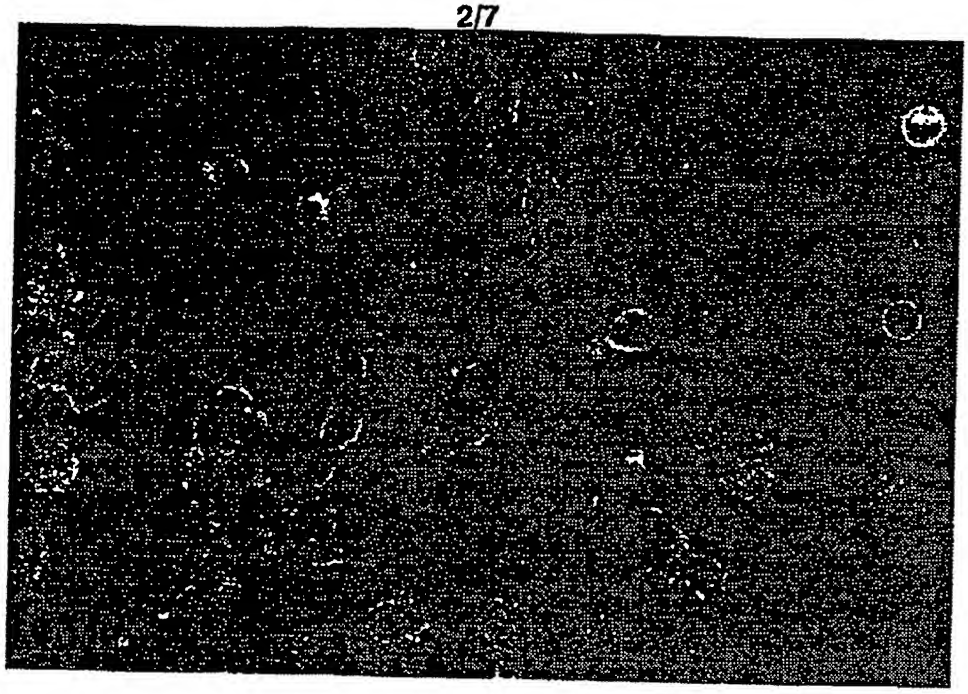
Fig. 1



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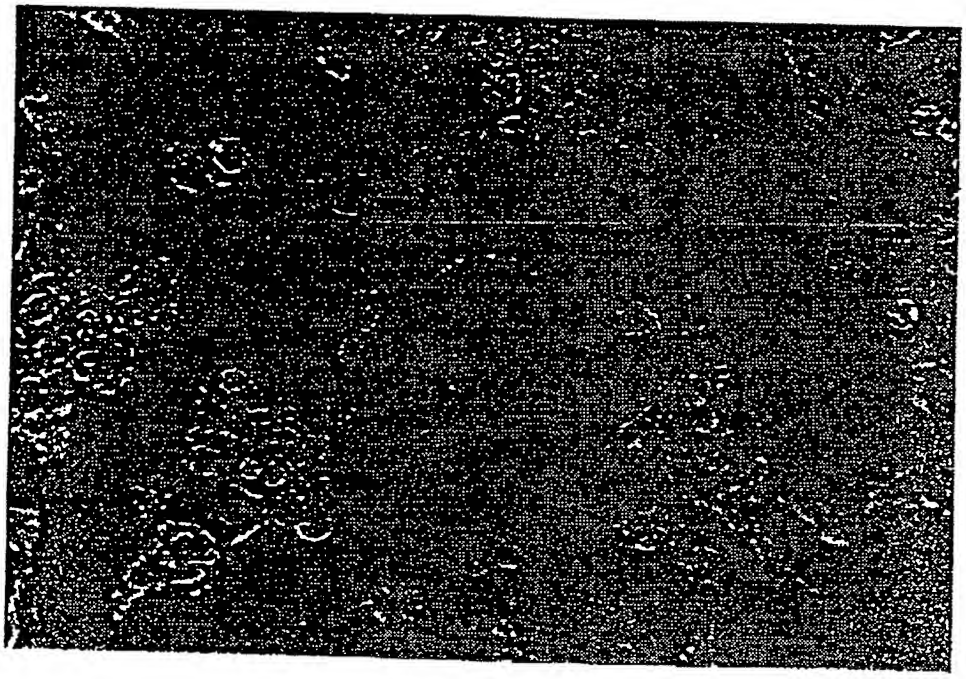

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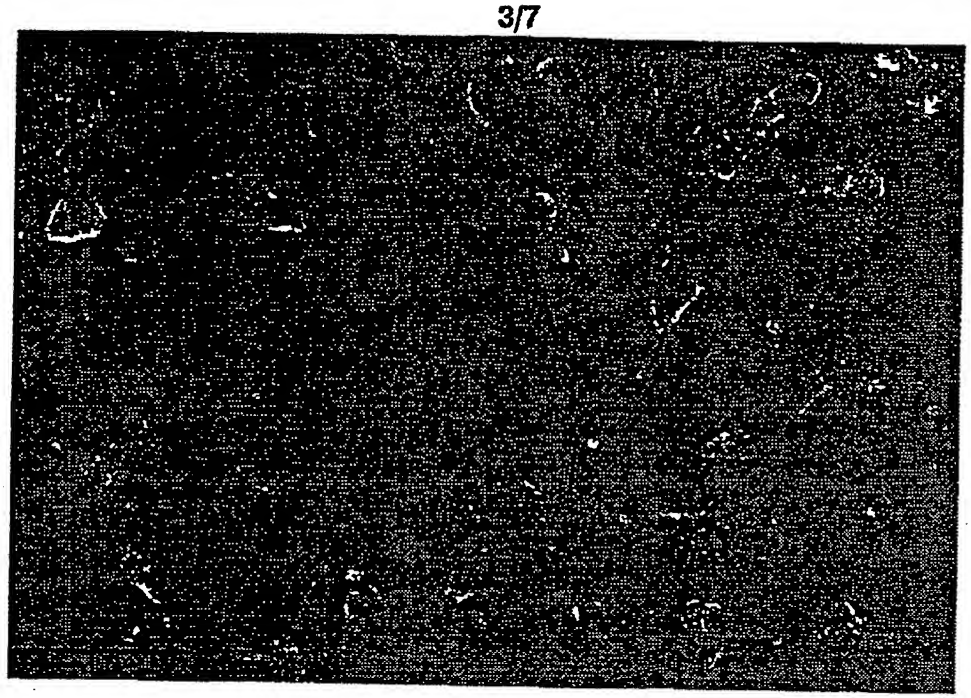
Fig. 2



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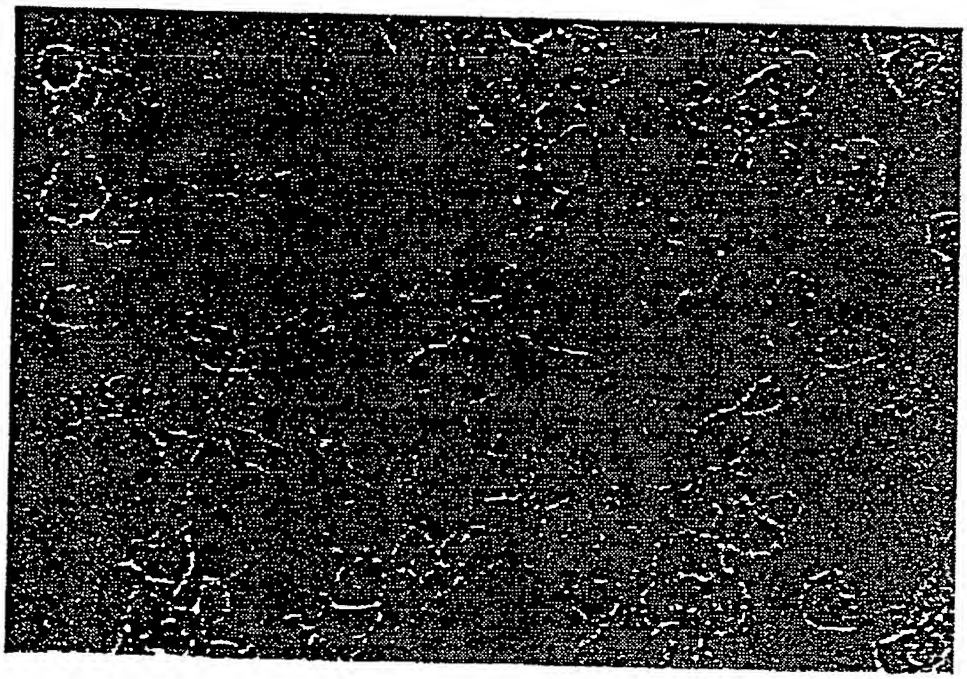

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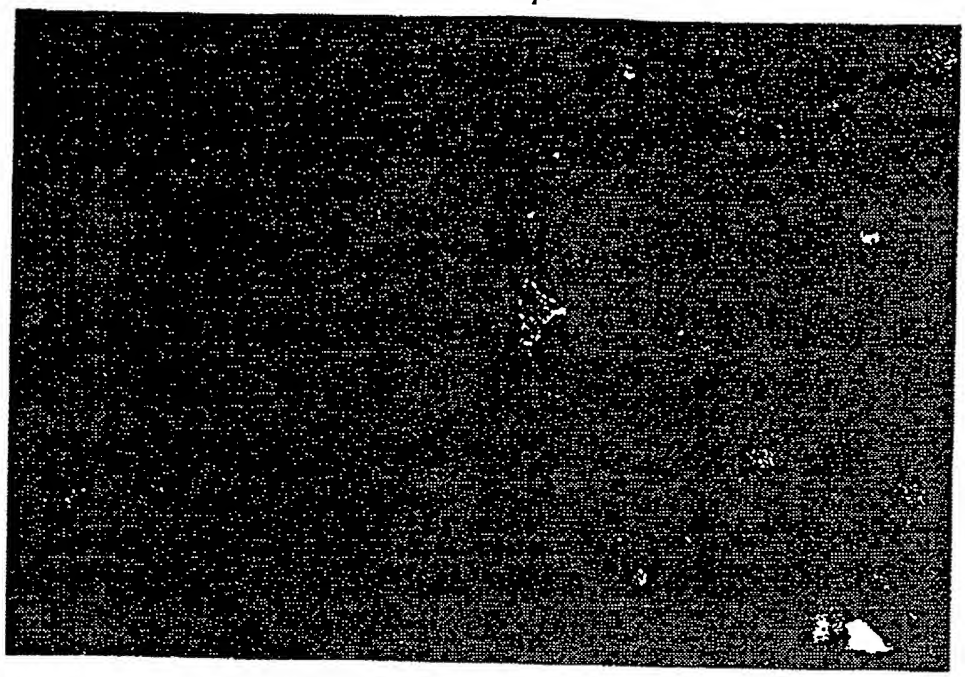
Fig. 3



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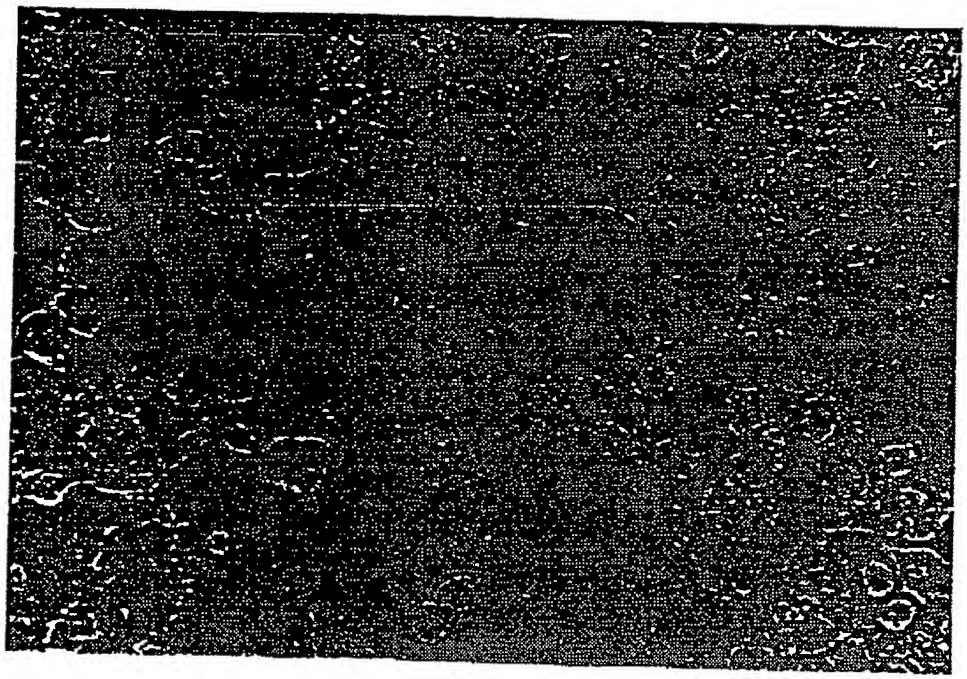
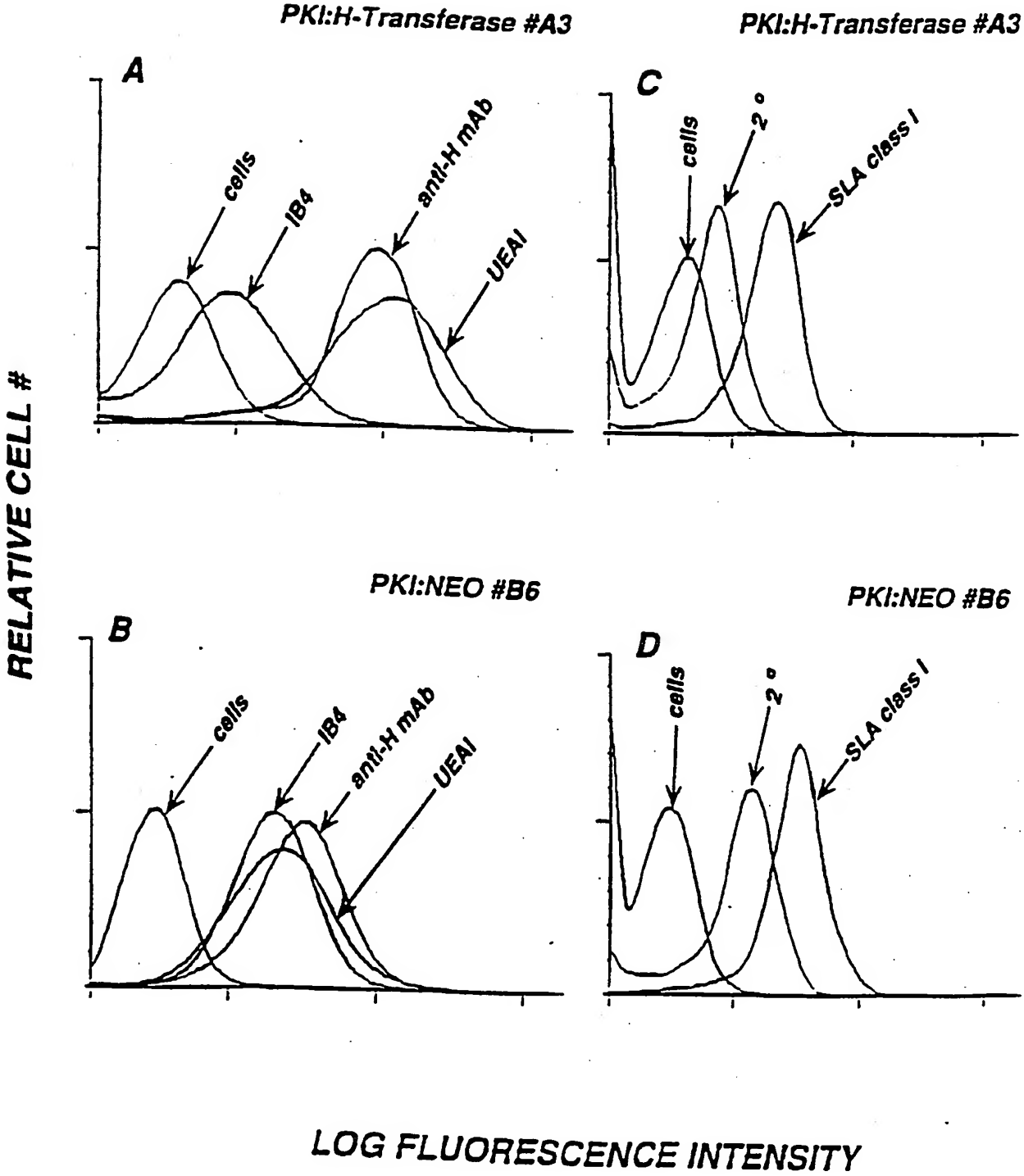


PC

Fig. 4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A
cells
IB4
anti-H mAb
UEAI
PKI:H-Transferase #A3
C
cells
2°
SLA class I
PKI:NEO #B6
B
cells
IB4
anti-H mAb
UEAI
PKI:NEO #B6
D
cells
2°
SLA class I
RELATIVE CELL #
LOG FLUORESCENCE INTENSITY

Fig. 5

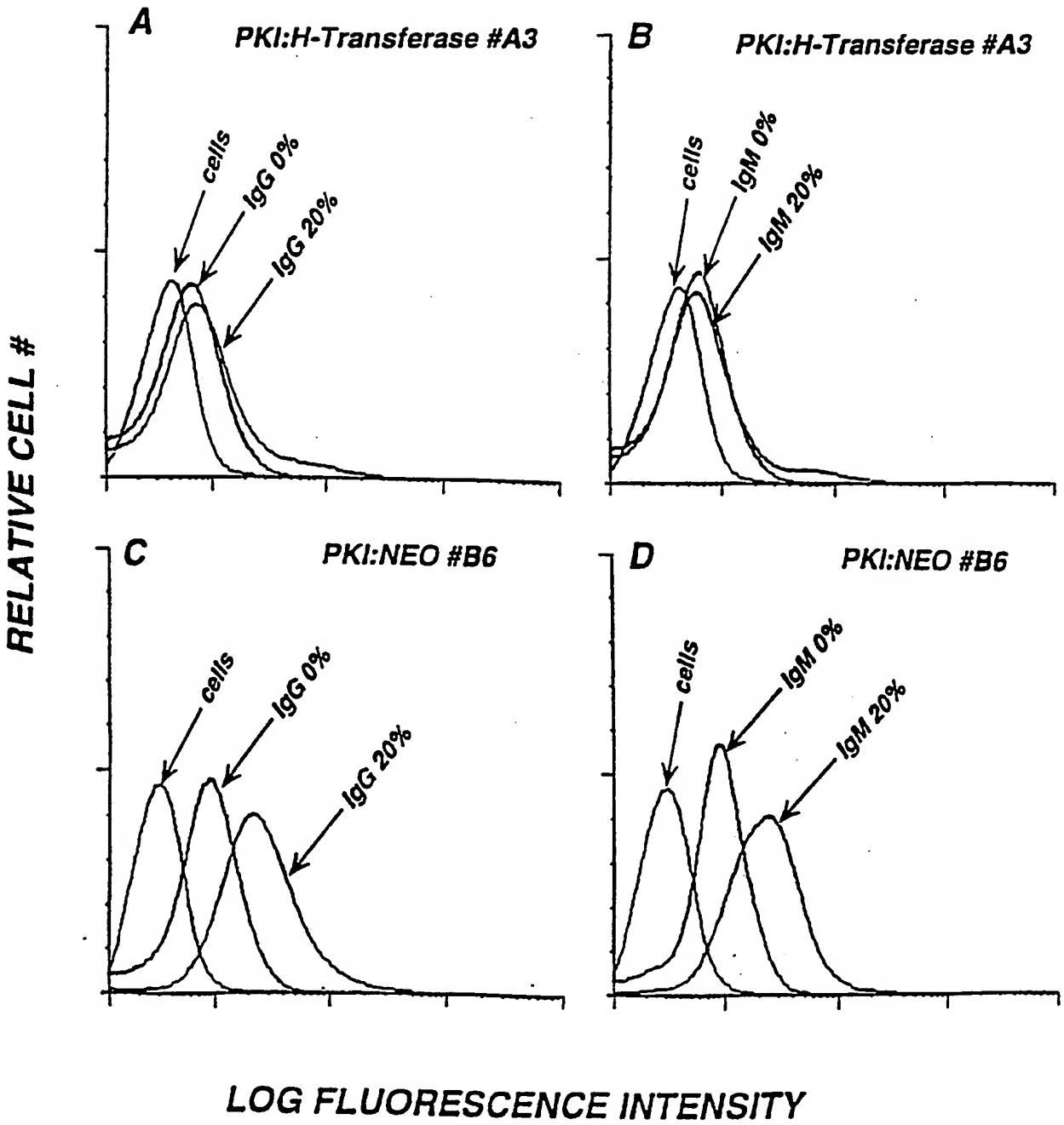
A
PKI:H-Transferase #A3
cells
IgG 0%
IgG 20%
B
PKI:H-Transferase #A3
cells
IgM 0%
IgM 20%
C
PKI:NEO #B6
cells
IgG 0%
IgG 20%
D
PKI:NEO #B6
cells
IgM 0%
IgM 20%
RELATIVE CELL #
LOG FLUORESCENCE INTENSITY

Fig. 6

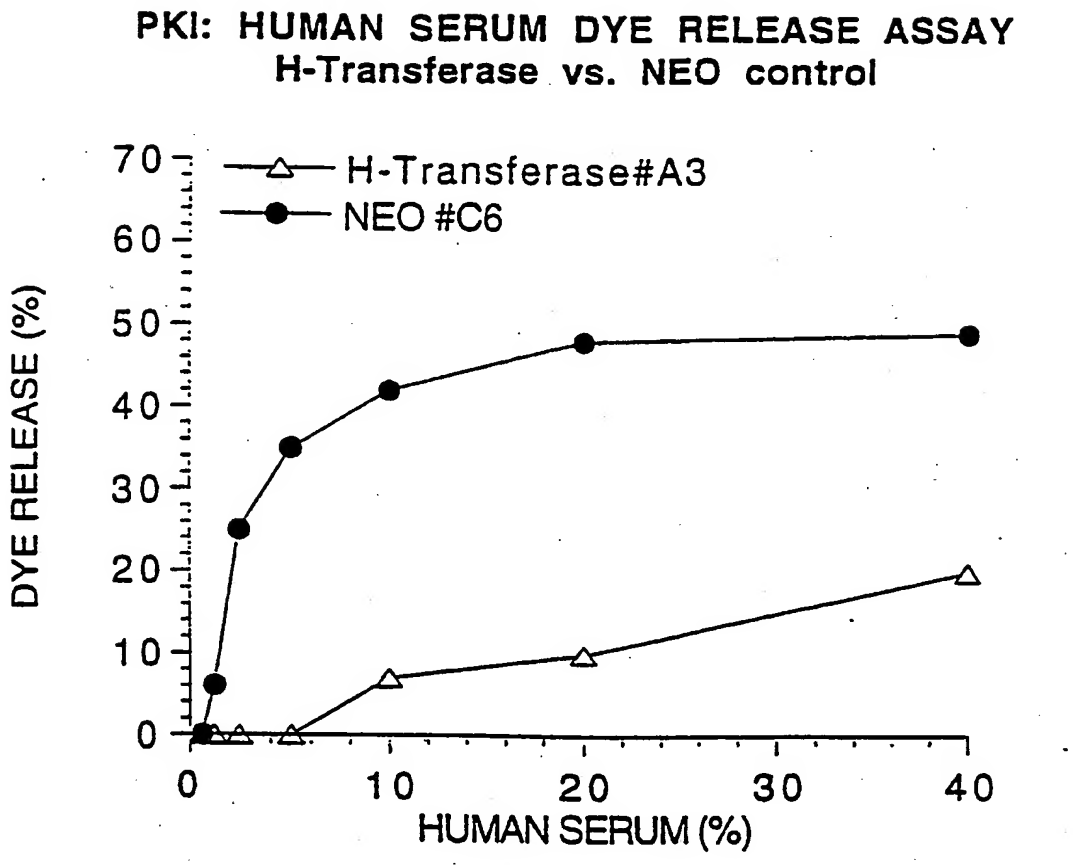
PKI: HUMAN SERUM DYE RELEASE ASSAY
H-Transferase vs. NEO control
H-Transferase#A3
NEO #C6
DYE RELEASE (%)
70
60
50
40
30
20
10
0
0
10
20
30
40
HUMAN SERUM (%)

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07554

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 43/04, 63/00; A61K 31/70, 48/00; C12N 15/00
US CL :424/93.21; 435/172.3, 320.1; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/93.21; 435/172.3, 320.1; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,283,058 (FAUSTMAN) 01 February 1994, see entire document.	1-7
Y, P	Immunological Reviews, Volume 141, issued October 1994, Gustafsson et al., "Alpha-1,3-galactosyltransferase: a target for in vivo genetic manipulation in xenotransplantation", pages 59-70, see entire document.	1-7
Y	Transplantation, Volume 56, Number 6, issued December 1993, Oriol et al., "Carbohydrate antigens of pig tissues reacting with human natural antibodies as potential targets for hyperacute vascular rejection in pig-to-man organ xenotransplantation", pages 1433-1442, see entire document.	1-7

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 AUGUST 1995	15SEP1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL NEWELL Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07554

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Lancet, Volume 342, issued 11 September 1993, Cooper et al., "Genetically engineered pigs", pages 682-683, see entire document.	1-7
Y	Transplantation, Volume 57, Number 5, issued March 1994, Ye et al., "The pig as a potential organ donor for man", pages 694-703, see entire document.	1-7
Y	Immunology Today, Volume 14, Number 10, issued 1993, Galili, "Interaction of the natural anti-Gal antibody with alpha-galactosyl epitopes: a major obstacle for xenotransplantation in humans", pages 480-482, see entire document.	1-7
Y	Transplantation, Volume 57, Number 10, issued May 1994, Kennedy et al., "Protection of porcine aortic endothelial cells from complement-mediated cell lysis and activation by recombinant human CD59", pages 1494-1501, see entire document.	1-7
Y, P	Transplantation Proceedings, Volume 27, Number 1, issued February 1995, Strahan et al., "Pig alpha-1,3-galactosyltransferase: Sequence of a full-length cDNA clone, chromosomal localisation of the corresponding gene, and inhibition of expression in cultured pig endothelial cells", pages 245-246, see entire document.	1-7
Y, P	Transplantation Proceedings, Volume 27, Number 1, issued February 1995, McKenzie et al., "Comparative studies of the major xenoantigen GAL-alpha (1,3)gal in pigs and mice", pages 247-248, see entire document.	1-7
Y, P	Transplantation Proceedings, Volume 27, Number 1, issued February 1995, Goldberg et al., "Inhibition of the human antipig xenograft reaction with soluble oligosaccharides", pages 249-250, see entire document.	1-7
Y	Proc. Natl. Acad. Sci. USA, Volume 90, issued December 1993, Sandrin et al., "Anti-pig IgM antibodies in human serum react predominantly with Gal (alpha 1,3) Gal epitopes", pages 11391-11395, see entire document.	1-7
Y	Transplantation, Volume 57, Number 6, issued March 1994, Fukushima et al., "The role of anti-pig antibody in pig-to-baboon cardiac xenotransplantation rejection", pages 923-928, see entire document.	1-7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07554

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	Veterinary Immunology and Immunopathology, Volume 43, issued 1994, Sachs, "The pig as a potential xenograft donor", pages 185-191, see entire document.	1-7

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, Medline, Embase, CAPlus
search terms: Sandrin, Fodor, Squinto, Rother, McKenzie, xenograft, xenotransplant, xenogeneic, xenoantigen, transplant, fucosyltransferase, galactosyltransferase, glycosyltransferase, ungulate, pig, porcine, hyperacute rejcetion, (gene, genetic, DNA, nucleic acid)(W)(therapy or delivery or transfer or treatment)

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